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Mechanisms to Detoxify Selected Organic Contaminants in Higher Plants and Microbes, and Their Potential Use in Landscape Management

LETTER REPORT

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ABSTRACT

In this report biochemical and physiological processes important for the phytoremediation of organic contaminants are discussed. Literature data on the uptake and potential of plants and microorganisms to detoxify organic contaminants are analyzed. Mechanisms of penetration and translocation of organic contaminants in plant leaves and roots are described. The principal transformation phases of organic contaminants once entered into a plant are: functionalization, conjugation and compartmentation. The following mechanisms to detoxify organic contaminants are discussed and the chemical reactions involved described: excretion, hydroxylation, reduction, hydrolysis, conjugate formation (glycosylation, peptide conjugation). The most important enzymes that participate in these processes, i.e., cytochrom P450-containing monooxygenase, peroxidase, phenoloxidase, nitroreductase, esterase, transferase, are characterized and known mechanisms of their actions discussed. Recently generated, so far unpublished, data on the uptake and transformation of TNT in plants and microorganisms are presented. Several effects on the plant cell ultrastructure are illustrated of organic contaminants with different chemical characteristics, as well as the changes cells undergo during the detoxification process. Two lists are presented of plant and microbial strains potentially utilizable for the remediation of organic contaminants. Further study of plants and microorganisms with a high potential for remediation of organic contaminants is recommended, and the subsequent application of the generated results is suggested.

Key words: Organic contaminants; Absorption; Detoxification; Higher plants; Microorganisms

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1 - INTRODUCTION

The authors are committed to the idea that our planet could benefit significantly more from its ecological potential, when the detoxification potential of plants and microorganisms would be recognized and effectively used worldwide. The search for, and subsequent identification of plant and microbial species, that effectively absorb and degrade organic contaminants may lead to new criteria as a basis for genetically engineered organisms with apparent industrial application potential.

The message of this review is that while a developing society coincides with the man-made creation of new chemicals, the application of relevant scientific knowledge to exploit the ecological potential of plants and microbes in new ways may enable the reversal of the seemingly unavoidable deterioration of our environment.

The last two hundred years — that is, since the beginning of large-scale industrial production of chemicals — have been marked by an increasing deterioration of the environment. Currently, more than 300 million tons of chemicals of different classes are synthesized annually, to which the pesticides alone contribute 640 thousand tons. Most toxic compounds (herbicides, insecticides, fungicides, acaricides, etc.) eventually accumulate in various environmental compartments (soil, water, air), and the majority affects the biota. These chemicals are also highly diverse. The majority of the synthesized chemicals (96%) is of organic nature, among which monocyclic compounds prevail (74%). The number of chemicals for general consumption is approximately 6500, with over 90% being used as food conservatives, dyes, aromatic compounds, emulgators, and pharmaceuticals. Current important sources of aggressive and intensive pollution are chemicals used in agriculture, industry, transportation, the production and transportation of oil, and military activities.

Recently increased attention has been devoted to the man-made use of the natural detoxification processes. Microorganisms have been used successfully for the purification of sewage and soil, and this technology effectively competes with abiotic technologies.

Plants, that occupy approximately 47% of the land surface, are capable to purify air, water, and soil, and –thus- have the potential to serve as universal detoxifiers. In any given ecosystem, typically 90% of the biomass consists of plants. The role of plants and microorganisms in maintaining a tolerable environment is illustrated by the following example. The burning of fuels for heating, the generation of electricity and automotive transport, formerly considered to be a fairly harmless activity, would release carbon oxides and other chemicals into the environment in sufficient quantities to exterminate all life, if green plants did not absorb and metabolize carbon dioxide and other chemicals to such an extent that the atmosphere can be tolerated by biota, including humans.

The universality of plants and microorganisms in remediation consists of their ability to absorb various organic chemicals from air, water or soil, and finally turn them into carbon dioxide. **As a result of such degradation, all carbon atoms of**

contaminants become part of the biogenic carbon cycle. If phyto- and bioremediation technologies are compared with existing chemical, physical and mechanical technologies, in which the filters or absorbents themselves turn into hotspots of contamination due to incomplete combustion of organic mass, the advantages of plant- and microbial based ecotechnologies as the most cost-effective are obvious.

Currently sufficient information is available to make rough estimates of the detoxification potential of plants. In this review existing data are analyzed to expose the ability of plants to assimilate and metabolize organic contaminants. Enzymatic systems participating in consecutive oxidative transformations of organic toxicants are described, and common patterns in plant responses upon exposure to exogenous organics in terms of changes in structure and resistance of the plant cell are elucidated.

Plant or microbial cells are not small factories in which organic contaminants are continuously absorbed and degraded. The limitations of these processes are directly related to the deviations from normal organization induced in these cells (especially plant cells) upon exposure. Depending on the concentration, exposure duration, contaminant type and environmental factors, the action can be avoidable (metabolizable) or lethal.

In this review special attention is paid to biological treatment of explosives-contaminated soils. This is done because of a perceived interest within the U.S. Department of Defense in low-cost applications of alternative remediation and management technologies for large areas contaminated by military relevant compounds. Explosives are divided into three general categories, according to their structure and functional groups: nitroaromatic compounds, nitrate esters, and nitramines. Nitroaromatic compounds constitute a major class of environmental contaminants. They have wide use as industrial feedstocks due to the versatile chemistry of their nitro- group. For instance, 97% of nitrobenzene produced worldwide is used for the production of aniline of approximately 3,0 billion pounds.

This review concerns mechanisms in higher plants and microbes to detoxify selected organic contaminant, and emphasizes the mechanisms potentially important for the remediation of contaminated areas on a landscape scale.

2 - ABSORPTION AND TRANSPORT OF ORGANIC CONTAMINANTS IN PLANTS

Organic contaminants enter plant cells from soil, water, and air, with sites of entry being roots and leaves. Xenobiotics enter roots together with water and nutrients. They enter leaves as a result of crop treatment with agrochemicals and by absorption of gaseous compounds from the air. Xenobiotics are absorbed less selectively by roots than by leaves.

Absorption by Roots

Organic contaminants pass into roots in a way that differs greatly from that into leaves. These substances can pass relatively freely into roots through cuticle-free unsuberized cell walls. Therefore, roots absorb substances far less selectively than leaves.

Organic compounds enter the roots together with water, similar to nutrients. They move towards the transport tissue (xylem) mainly along the apoplast, a system of intracellular spaces. Relatively few organics move along the symplast, composed by plasmodesmata bridging cells. The compounds penetrate into the apoplast, by diffusion, andmove easily through these capillaries without meeting membrane barriers on their way. In contrast, during symplastic transport, many membrane barriers are met.

Organics are absorbed by roots in two phases (Korte et al. 2000). In the first phase, they diffuse rapidly from the surrounding medium into the root. The absorption rate is directly proportional to the concentration of the compound in the soil or nutrient solution. In the second phase, they accumulate slowly in the tissues. The intensity of the absorption process depends on the molecular weight of the compound, concentration, polarity, pH, temperature, soil humidity and other factors (Ugrekhelidze et al. 1986; Kristich, Schwarz, 1986). In the initial absorption phase of organics, diffusive penetration of substances into the roots apoplast takes place. The absorption rate is directly proportional to the concentration of the compound in the soil or nutrient solution.

Temperature strongly influences the absorption of organics by roots. A 10^{o}C increase in temperature increases diffusion only by a factor of 1.2 to 1.4. However, the subsequent active transport is controlled by transpiration, metabolic activity and accumulation, and in the latter case, a 10^{o}C increase in temperature increases transpiration and enzymatic reactions by a factor of 1.3 to 5.0 (Korte et al. 2000).

The molecular weight of a compound is the main limiting factor during the passage of organics into roots. Plants easily absorb organic compounds with molecular weights $\leq$1000 (Söchtig, 1964). Studies on polyethylene glycol absorption by roots of cotton (*Gossypium hirsutum*; Lawlor, 1970) and pepper (*Capsicum annuum*; Janes, 1974) have shown that larger molecules also penetrate the roots. For example, small amounts of polyethylene glycol with molecular weights between 4 000 and 20 000 were found in plants. The amount of polyethylene glycol entering into the plant was inversely proportional to the polymer molecular weight. It should be mentioned that polyethylene glycol enters plants much faster and in significantly greater amounts if the roots are damaged. Polyethylene glycol absorbed by kidney bean and cotton seedlings is translocated through the plant without changing its molecular characteristics (Andreopoulos-Renaud et al. 1975). According to other data, high molecular weight compounds can be absorbed by roots only after partial degradation of the molecules (Führ, Sauerbeck, 1974).

The majority of the (^{14}C labelled) high molecular weight humic acids is adsorbed on the surface of roots and partly penetrates into the cells of epidermis (this has been demonstrated in sunflower (*Helianthus annuus*), wild radish (*Raphanus sativus*) and wild carrot (*Daucus carota*)). Smaller molecules of fulvic acids penetrate more deeply and reach the central cylinder of the xylem. However, the labelled carbon of fulvic acids does not penetrate into the aboveground plant parts. Experiments with polyurethane have shown that the labelled carbon polymer is absorbed (after preliminary partial degradation of the polyurethane molecules in the soil) by tomato (*Lycopersicum esculentum*), cucumber and strawberry (*Fragaria vesca*) root systems (Führ, Mittelstaedt, 1974).

Roots absorb a wide spectrum of hydrophilic and lipophilic organic molecules (aliphatic, aromatic and hydroaromatic hydrocarbons, alcohols, phenols, amines, etc.). Even substances with an extremely low solubility in water, such as polycyclic hydrocarbons, are absorbed (Dörr, 1970; Devdariani, Kavtaradze, 1979). The absorption process greatly depends on the lipophilicity of the compound. Substances with a moderate hydrophilicity, i.e. a log K_{OW} (n-octanol/water partitioning coefficient) of 0.5 to 3.0, are most actively absorbed (Korte et al. 1992).

The absorption process in roots is also affected by pH of the soil or nutrient solution. For instance, the amount of absorbed insecticide picloram by the roots of oats (*Avena sativa*) and soybean (*Glycine max*) sharply decreased when the medium pH was changed from 3.5 to 4.5, whereas a further change in the range of 4.5–9.5 influenced absorption insignificantly (Isensee et al. 1971). At pH 3.5 only 20% of the picloram is ionized but at pH 4.5 the degree of ionization reaches 71.5%. Therefore, it was concluded that the insecticide is absorbed by plant seedlings predominantly in nonionic form. Many organic compounds are predominantly assimilated as undissociated molecules by roots and leaves, i.e. without charge (Ugrekhelidze et al. 1986). Desorption of organics from soil particles and subsequent transport in soil also depends on pH. For example, the herbicide atrazin is more extractable from alkaline soil (pH 8.3), but other herbicides, such as chloramben and dicamba, are more extractable under weakly acid conditions (pH 4.1; Lavy, 1975).

Since the organic compounds enter the roots in soil solution, the rates with which they are absorbed by the root system are also determined by soil moisture content. Consequently, the absorption of organics by plants decreases with decreasing soil moisture content. Soil moisture also affects desorption of organics from soil. The extractability of symmetric triazines, atrazine and chloroamben from soil by organic solvents is considerably enhanced by the addition of water (Lavy, 1975).

Soil organic matter (e.g. humic and fulvic acids) also influences absorption of organic compounds by roots. For instance, the toxicity of the herbicides prometryn, fluometuron and trifluralin decreased with the increase of organic matter content in soil (Weber et al. 1974). Similar results were obtained in a study of thirteen representatives of symmetric triazines (chlorine, methylthio- and methoxytriazine) with oat seedlings (Rahman, Matthews, 1979). It was also found that the toxicity of hydrophilic toxicants decreased to a lesser degree than that of lipophilic ones. It was, therefore, concluded that

the increase in soil organic matter content promotes the adsorption of lipophilic herbicide molecules to soil, impedes the herbicide absorption by roots, and prevents the herbicides to attain a toxic concentration in the plant cells.

Transpiration, metabolism, and mineral nutrition of plants are significantlyinfluence the absorption of organics by roots. For example, urea not only stimulates transpiration but also the absorption of atrazine in tomato roots (Minshall et al. 1977). Vice versa, inhibitors of respiration such as cyanide, at a concentration of 10^{-3} M, may reduce the absorption of trichloroacetic acid in wheat and oat seedlings by approximately 30%. 2,4-Dichlorophenol (2,4-D), a common inhibitor of metabolism at a concentration of 10^{-3} M, suppresses the absorption of trichloroacetic acid by 70% and 54% in wheat and oat seedlings, respectively (Chow, 1970). The presence or absence of nutritional elements affects the absorption of organics by roots in a different way. The absorption of methyl-2-benzimidazolecarbamate and methylthiophanate by kidney bean seedlings is reduced by deficiencies in nitrogen, sulfur and boron in the nutrient solution. However, the absorption of parathion is increased by deficiencies of nitrogen, boron or potassium in the same plant (Al-Adil et al. 1974). In contrast, the absorption of the herbicide buturon by wheat roots is decreased by deficiencies in nitrogen, phosphorous, potassium or magnesium (Haque et al. 1977). Moreover, the absorption of the fungicides benzimidazole and thiophanate by isolated maize roots (*Zea mays*) was not affected by mineral salts (Leroux, Gredt, 1975).

Absorption by Leaves

In order to penetrate into a leaf, the organic xenobiotic should pass through the stomata, or traverse the epidermis, covered by the cuticle. Stomata are usually located on the lower (abaxial) side of a leaf, and the thicker cuticular layer on the upper (adaxial) side. The stomatal system consists of numerous apertures, which may be resized when required, and regulates the penetration of organics in the leaves. By changing the diameter of the aperture the plant controls the entry of compounds of different molecular masses. Opening and closure of the stomata is controlled by the movement of two guard cells, that are modified epidermal cells (Libbert, 1974).

The movement of these guard cells is regulated by the concentration of potassium ions – stoma being opened by an increase in K^+ concentration. The degree of stomatal aperture opening depends on external environmental conditions, such as light, temperature, humidity etc., and on internal factors such as the partial carbon dioxide pressure in the intracellular spaces, the degree to which the plant is hydrated, ionic balance and the presence of pheromones (Libbert, 1974).

Gases and liquids penetrate the leaves through stomata. The permeability for gases depends on the degree of opening of the stomatal apertures (4–10 nm), and the permeability for liquids depends on the degree of moisture of the leaf surface, the surface tension of the liquid and the morphology of the stomata. The majority of organic compounds penetrate into a leaf as a solution (pesticides, air pollutants, liquid aerosols etc.). The crossover surface tension of in the lower surface of leaves of *Zebrina purpusii*

was 25–30 dyne cm^{-1} (for comparison: the surface pressure of water is 72.5 dyne cm^{-1} and for ethanol 22 dyne cm^{-1}) (Schönherr, Bukovac, 1972). Liquids with a surface tension <30 dyne cm^{-1} have a constant angle of contact with the surface of a leaf and instantly penetrate into the stomata. Liquids with surface tension >30 dyne cm^{-1} are able to penetrate into the stomata without moistening the leaf surface.

The penetration of α-naphthylacetic acid into the stomata–rich lower surface of pear (*Pyrus* sp.) leaves was considerably stimulatedby light (Greene, Bukovac, 1977). The growth regulator, α-naphthylacetic acid, and other natural phytohormones significantly influence the opening of the stomata. Experiments on isolated leaves of light-blue snakeweed (*Stachytarpheta indica* (L) Vahl) showed that α-naphthylacetic acid and its hydroxyderivative metabolite 2-naphthoxyacetic acid restrict, but do not prevent, the opening of stomata and suppress the accumulation of potassium in the guard cells (Pemadasa, 1979). The penetration of succinic acid 2,2-dimethylhydrazide through the surface of isolated leaves of kidney bean (*Phaseolus vulgaris)* supports the important role of the stomata for the absorption of organics by leaves (Schönherr, Bukovac, 1978).
However, evidence also exists indicating that the role of stomata in the absorption of organic compounds by leaves is not important. In addition, contrasting data for the same compounds have been published. For example, the presence of stomata on the leaf surface did not influence the penetration of the herbicide 2,4-D into the leaves of legumes, but the penetration of the herbicide into the leaf was increased by illumination. (Day, 1952). Experiments with various ecotypes of creeping thistle (*Cirsium arvense*), with distinct numbers of stomata per unit of the leaf area, have shown that the penetration of 2,4-D into leaves does not depend on the surface area of the stomatal apertures and the density of the stomata (Hodgson, Moore, 1972).

For inorganic gases penetration through the stoma is the main pathway. For example, carbon dioxide is absorbed 100 times more rapidly through stomata than through a cuticle. For plants in which stomata are lacking, i.e., ferns, aquatic plants, algae, etc., penetration through cuticle of epidermis is the sole path for toxicants enter to the leaves.

The cuticle consists of a thin wax layer covering almost all aboveground parts of higher plants, including the epidermis of the leaves. The cuticle is permeable not only for lipophilic substances, but also for hydrophilic molecules of gas, water and sulphuric acid (Libbert, 1974). The thickness and chemical composition of the leaf cuticle vary with species, age, location on the stem, and environmental factors such as temperature, humidity, etc. The wax of the cuticle (cutin) is a complex mixture of long-chain alkanes, alcohols, ketones, esters and carboxylic acids. Alkanes and esters predominate on the outside surface of a cuticle. Besides, long-chain C_{29}-C_{33} diketones, triterpenoids (for example, ursolic acid), diterpenes, glycerides and phenolic compounds are sometimes present in the wax. The main mass of leaf wax is attributable to normal long-chain alkanes with an odd number of carbonic atoms in the chain (C_{31}-C_{37}), in particular the n-alkanes $C_{29}H_{60}$ and $C_{31}H_{64}$, and esters of n-carboxylic acids with primary and secondary alcohols (Eglinton, Hamilton, 1963).

Cuticle thickness varies and depends not only on plant species, but also on the age of the leaf. Thickness is not always an indicator of wax content. For example, in two varieties of plum (*Prunus domestica*) the adaxial surface of leaves has a thicker cuticle (1.6-2.0 nm) than the abaxial one (1.2 nm), but the former contains less surface wax (densities 34-35 and 47-52 μg/cm^2 respectively) (Leece, 1978). In young leaves the cuticle is usually thinner and less uniformly developed than in old ones. The quantity of stomatal wax also increases with aging. The synthesis of cutin is terminated only after complete leaf greening (Kolattukudy, 1980). This explains why young leaves absorb toxic compounds much more intensively than adult leaves. For example, the absorption of succinic acid 2,2-dimethylhydrazide through the surface of kidney bean leaves decreased with leaf age (Schönherr, Bukovac, 1978). Similar results have been reported for 2,4-D, nitrophen, indoleacetic acid (IAA) and some other toxicants (Sargent, Blackman, 1972; Epstein, Lavee, 1977; Pereira et al. 1971).

The organic compounds adsorbed on the lipophilic surface of the leaf wax accumulate in the cuticle in large amounts and gradually penetrate into the leaf cells. The wax appears to be an active sorbent for lipophilic organic compounds (Bukovac et al. 1990). Apparently, the molecules of the adsorbed toxicants together with individual wax components migrate from the cuticle inside the epidermal cells and are incorporated into endocellular membranes (Cassagne, Lessire, 1975). The wax layer of a cuticle serves as a barrier for the adsorption and penetration of organic compounds into leaves. In isogenic lines of peas (*Pisum sativum*) with distinct genes determining wax formation this difference is expressed phenotypically in cuticle thickness. The pesticides carbophos and methylnitrophos rapidly penetrate into the leaves of wax-less plant lines, reaching the photosynthesizing tissues in 3-4 h (Novojhilov, 1977). These pesticides slowly penetrate in plants with leavescoated with wax. In another example, removal of wax from the surface of an apex of European furze (*Ulex europaeus*) seedlings caused a 3.5 fold increase in the absorption of ^{14}C-picloram (a systemic herbicide; Rolston, Robertson, 1975).

The ease with which an organic compound penetrates a cuticle depends largely on its physical properties and lipophilicity. For instance, in contrast to the pesticide pyrazone, which promptly penetrates into the leaves of red beet (*Beta vulgaris*), phenmedipham and benzthiazuron are absorbed in insignificant amounts (Merbach, Schilling, 1977). Many organic compounds are capable to change the composition and structure of the cuticular wax by increasing the permeability of the cuticle. For instance, dimethylsulphoxide penetrates very rapidly through a cuticle by due to it's surfactant nature (Jones, Foy, 1972). The cuticle is also permeable to large molecules, such as some surfactants, fatty acids, long-chain alkanes, peptides, salts of 2,4-D with long-chain amines, etc. (Eynard, 1974; Cassagne, Lessire, 1975; Shida et al. 1975). However, in the latter cases the correlation between permeability and molecular weight is poor.

The aggregation state of the organic compound is very important for the infiltration into leaves.. For the fungicide pyracarbolide, infiltration increased by enhancing the degree of dispersion in the following order: powder < suspension < emulsion < solution (Sachse et al. 1974).

The penetration of solubilized ionogenic herbicides into leaves largely depends on the pH of the herbicide solution, determining the degree of dissociation of weak acids and basis. An interesting regularity is observed with such toxic compounds as picloram, 2,4,5-T, succinic acid 2,2-dimethylhydrazide and α-naphthylacetic acid. Xenobiotic molecules, particularly weak acids, are predominantly absorbed in their non-dissociated state, since in this nonionic state they have a lower polarity and easily pass the lipophilic wax barrier of the cuticle (Simon, Beavers, 1954; Baur et al. 1974; Schönherr, 1976; Rolston, Robertson, 1975; Schönherr, Bukovac, 1978).

Organic compounds penetrate more easily into damaged plant tissues than into intact plants. For example, the amino acid derivative pesticide N-lauroyl-L-valine penetrated into the intact leaves and fruits of cucumber (*Cucumis sativus*) in far smaller amounts than in damaged plants (Shida et al. 1975). Potential pathways for the penetration of lipophilic organic compounds in leaves were demonstrated for the absorption of gaseous hydrocarbons in leaves with stomata only on the lower surface (Ugrekhelidze, 1976). In this case, the leaves of the field maple (*Acer campestre*), wild Caucasian pear (*Pyrus caucasica*), vine (*Vitis vinifera*) and narrow-leaved oleaster (*Elaeagnus angustifolia*) were placed in an atmosphere containing ^{14}C-methane or [1-6^{14}C] benzene. Contact with the ^{14}C-labelled hydrocarbon occurred only at one side of the leaf. The total radioactivity recovered from the nonvolatile metabolites formed indicated that the absorption of the gaseous alkanes and vapors of the aromatic hydrocarbons was initiated by the leaves not only through stomata, but also through cuticle.

Similar results have been obtained for a number of herbicides (α-naphthylacetic acid, 2,4-D, pycloram and derivatives of urea), applied in soluble form to leaves (Sharma, Vanden Born, 1970; SargeNt, Blackman, 1972; Leece, 1978). Organic compounds are absorbed more intensively by the abaxial side of a leaf, rich in stomata, than by the adaxial side. These results imply the active participation of stomata in the absorption of organic xenobiotics.

The cells of trichomes (outgrowths of the epidermis as filaments, warts, scales, setas etc.) can also participate in the absorption of toxic compounds. The number of radial trichomes on the adaxial surfaces of leaves has been correlated with the absorbed herbicide ^{14}C-triclopyr using young leaves of tanoak (*Lithocarpus densiflorus*; King, Radosevich, 1979).

Another mechanism by which organic compounds may enter leaves is through the ectodesmata, hollow organelles in cell walls filled by canals consisting of cellulose fibrils. These canals connect the plasmalemma to a cuticle and can serve as conductive pathways both during the absorption of water-soluble substances by a leaf and during their excretion. For example, adsorption of the herbicide 2,4-D (tritium-labeled) takes place on the anticlinar walls of the epidermal cells, mainly at sites with ectodesmata, and the absorption of 2,4-D by the leaves of wheat and kidney bean is directly proportional to the number of ectodesmata per unit area of the epidermis surface (Franke, 1975).

Translocation of Organic Contaminants in Plants

Organic xenobiotics absorbed by roots and leaves are translocated into different organs of the plants as a result of the physiological processes transporting nutrients. The main forces are:

- Transpiration streaming: transport of water and dissolved substances from roots to shoots, passing through vessels and tracheids located in the xylem.
- Assimilate streaming: transport of assimilates from leaves to the plant parts located below (shoot axis, root) and above (apex, fruits) the leaves, passing through sieve tubes located in the phloem.

Transpiration protects the plant from overheating. Stomata and cuticles participate in this process. The majority of the water is evaporated into the atmosphere via the stomata;.Cuticular transpiration typically comprises not more than 10%; in plants with a thin cuticle it may reach 20%. The total area of the stomata apertures constitutes only 1–2% of the leaf surface, but even this comparatively small area enables the evaporation of a significant amount of water. For instance, an average birch tree (*Betula*) evaporates approximately 400 l of water per day, poplar (*Populus sp*) from 190 to 1330 l, willow (*Salix alba*) 1900 l, which is about same amount evaporated from alfalfa (*Medicago sativa*) occupying 0.243 ha (Gatliff, 1994). The daily transpiration stream depends on changes of temperature: the maximum flux occurs soon after noon and the minimum at night. This process is driven by the opening and closing of stomata under the influence of sunlight during the day and its absence at night. During hot, dry days the evaporation rate of water from the leaf surface exceeds the flow of water from the roots. The resulting water deficiency leads to leaf deformation, which tends to close the stomata, leading to a decrease of transpiration rate. At night the stomata are closed in the absence of illumination, the ambient temperature falls and the water evaporation rate decreases, and water deficiency in the roots does not occur.

The importance of the transpiration stream for the absorption and translocation of organic compounds by plants is expressed in the following equation (Schnoor, Dee, 1997):

$$U = (TSCF)\,(T)\,(C)$$

where: U is the rate of organic compound assimilation (mg day^{-1}); T the rate of plant transpiration, (l day^{-1}); C the organic compound concentration in the water phase of soil (mg l^{-1}); *TSCF* the transpiration stream concentration factor, dimensionless, showing the ratio between the concentrations of organic compound in the liquid of the transpiration stream and in the environment (Paterson, Mackay 1990). The TSCF depends on the physical and chemical characteristics of the organic compound and can be estimated by the empirical equation provided below (Burken, Schnoor, 1997):

$$\mathrm{TSCF} = 0.75 \exp\left[-\frac{(\log \mathrm{K_{ow}} - 2.50)^2}{2.4} \right]$$

However, plant tissue levels of selected organic pollutants such as explosives (e.g TNT) absorbed from field soils have not been predicted successfully following this approach (Best et al., 2004) because uptake and transformation processes have been ongoing for extended periods of time.

The main parameter characterizing the organic compound in the empirical equation is K_{ow}, the partitioning coefficient between octanol and water. The K_{ow} gives an indication of the hydrophobicity, which predetermines the effectiveness of absorption and translocation of an organic compound in plants. It is known that compounds with a $\log K_{ow} > 3.5$ adsorb easily on soil grains or plant root surfaces and do not penetrate into the interior of the plant. Examples of such compounds are 1,2,4-trichlorobenzene, 1,2,3,4,5-pentachlorophenol, PAHs, PCBs, dioxins, etc. Moderately hydrophobic toxicants with a $\log K_{ow}$ between 1 and 3.5 (phenol, nitrobenzene, benzene, toluene, trichloroethylene, atrazine, etc.) are absorbed in large quantities and more easily penetrate into the plant. Hydrophilic toxicants with a $\log K_{ow} < 1$ (aniline, hexahydro-1,3,5-trinitro-1,3,5-triazin (the explosive RDX), etc.) are slightly adsorbed and not intensively assimilated by plants (Schnoor, Dee, 1997).

The transpiration stream passes through the xylem, and the assimilate stream through the phloem. Transport through the xylem is unidirectional, i.e., from the roots to the shoots, whereas transport through the phloem can be bi-directional, i.e. basipetally and acropetally. Organic compounds translocated in roots can migrate to the xylem through the apoplast in the following way: Root hairs → intracellular spaces → cell walls of cortical cells → endodermis → diffusion through the casparian strip (suberized barrier) → xylem. If organic compounds translocate inside the plant via the transpiration stream after penetration, the xenobiotics absorbed by the leaves are translocated together with assimilates formed in leaves.

The pH of the cell cytoplasm varies from 7.0 to 7.5, the pH of phloem sap about 8.0, and the pH of the vacuoles 5.5. The pH of the apoplast, including the intracellular space and the xylem vessels, ranges from 5.0–6.0. Water flows 50 to 100 times faster in the xylem than in the phloem, the latter being 0.5 to 1.0 m h-1 (Bromilow et al. 1990).

The assimilate streaming originates as follows: a high osmolyte concentration at the site of assimilate formation enhances the osmotic absorption of water and induces a high hydrostatic pressure. At the same time a low osmolyte concentration occurs in the neighbouring cells where the assimilates are excreted, insignificant osmotic absorption of water and a weak hydrostatic pressure. The flow of solution through the semi-permeable membrane between the sieve tubes and the surrounding cells in the direction of the concentration gradient levels the pressure.

Organic compounds can bind with soil particles reversibly or irreversibly, depending on the hydrophobicity of the toxic compound (the K_{ow} value). Part of the compound can undergo transformation by microorganisms in the plants' rhizosphere, the other part that penetrates into the plant roots can migrate into the xylem. Degradation

products of the organic compound, resulting from microbiological destruction, can also enter the plant roots. After absorption, the organic compound is translocated through the transpiration stream and distributed throughout the plant. Translocation pathways of organic compounds in plants are supported by a substantial body of experimental data. Plants exposed to low concentrations of C_1–C_5 alkanes, cyclohexane, benzene, and toluene absorb these substances and degrade them by deep oxidation. Results of experiments in which various plant species (55 annual and perennial plant species) were incubated with ^{14}C-labelled hydrocarbons indicate that all tested plants absorb and transform alkanes and aromatic compounds with different rates (Durmishidze et al. 1974; Durmishidze, Ugrekhelidze, 1975). The degradation products of the hydrocarbons initially assimilated by the leaves were transported through stems to roots, but hydrocarbons absorbed by the roots were transformed and transported to the leaves (Ugrekhelidze, Durmishidze, 1984).

Many polycyclic aromatic hydrocarbons (PAHs) are actively absorbed and transported byroots and leaves from the nutrient solutions, despite their high hydrophobicity (Muller, 1976; Devdariani, Kavtaradze, 1979; Devdariani, 1988; Holoubek et al. 2000). PAHs containing two or three aromatic rings with a low molecular weight, e.g. naphthalene, anthracene, and phenanthrene, are absorbed and degraded more easily than PAHs with high molecular weights, e.g. perylene, 3,4-benzoapyrene, benzanthracene, and dibenzanthracene.

Aryloxycarbonic acid pesticides penetrate the leaf cuticle in the form of undissociated molecules and are absorbed by the parenchymal cells. These compounds reach the phloem via the symplast, enter the sieve tubes, and are thus transported to the leaves, actively growing tissues and reproductive organs. The herbicide 2,4-D and defoliant 2,4,5-T are absorbed by leaves and translocated basipetally and acropetally in kidney bean (Long, Basler, 1974). The herbicide mecoprop is abosorbed by leaves and transported only basipetally in both sensitive and resistant biotypes of common chickweed (*Stellaria media*; Coupland et al. 1990).

Pesticides derived from urea are easily absorbed from nutrient solution by the plant root system and most of them are rapidly translocated acropetally in the plant with the transpiration stream. ^{14}C-fluometuron was absorbed from the nutrient solution by the roots and rapidly translocated acropetally by the transpiration stream in cotton and kidney bean seedlings. In cases where this herbicide is absorbed by leaves, it may move basipetally and acropetally, confirming the symplastic translocation of this herbicide along the phloem (Rubin, Eshel, 1977). Another urea derivative, the herbicide tebuthiuron, follows a similar migration route (Steinert, Stritzke 1977). Chlorimuron applied to the leaves of soybean (*Glycine max)*, peanut (*Arachis hypogaea*) and other weeds showed slow symplastic and apoplastic migration (Wilcut et al. 1989). It is interesting to note that this herbicide once absorbed by roots of yellow (*Cyperus esculentus*) and purple nutsedge (*Cyperus rotundus*) is transported acropetally, but is not transported when absorbed by tubers (Reddy, Bendixen, 1989).

Carbaminate pesticides are typically trasnlocated acropetally. Examples are carbofuran in seedlings of soybean and mung bean (*Vigna radiata*) (Talekar et al. 1977), methyl-2-benzimidazole carbaminate in seedlings of peanut (Vias et al. 1976; Prasad, Ellis, 1978) and safflower (*Carthamus tinctoria*; Mathur, Jhamaria, 1975). Phenmedipham and desmodipham, absorbed by the leaves of wild mustard (*Brassica kaber*), redroot pigweed (*Amaranthus retroflexus*) and sugar beet (*Beta vulgaris*), were also translocated only acropetally (Hendrick et al. 1974).

The direction in which the translocation occurs has been found to depend on plant resistance towards the toxic compound. For instance, the herbicide buthidazole is translocated acropetally and basipetally after absorption by the leaves of the sensitive amaranth, , but transported only basipetally after absorption by leaves of the resistant maize (Hatzios, Penner, 1980). The latter herbicide is barely translocated along the apoplast in soybean leaves (Haderlie, 1980). 4,4´- methylene-*bis*-(2-chloraniline) is absorbed but not translocated after application to the leaves of different plants (Bromilov et. al. 1990).

Tyree (1979) formulated the hypothesis of intermediate permeability for the transport of xenobiotics through the phloem is the mechanism underlying the hypothesis of intermediate permeability suggested by Tyree (1979). This hypothesis takes the proximity of phloem and xylem vessels into account, and proposes that:

- Any molecule with a high membrane permeability will be able to enter the phloem, but can also leave the phloem and be more rapidly transported to the xylem;
- Any molecule with a low membrane permeability can not attain a sufficiently high concentration in the phloem to be effectively transported;
- An intermediate permeabilityshould exist, and compounds with an intermediate permeability must be characterized by the highest mobility in the phloem.

Based on this hypothesis and based on a data set on herbicide assimilation and transport by castor bean seedlings, Kleier and others developed a mathematical model describing the translocation of organic compounds in plants (Hsu et al. 1988; Grayson, Kleier, 1990; Hsu, Kleier, 1990; Kleier, 1994; Brudenell et al. 1995). Kleier's model has been successfully used to predict translocation of many secondary metabolites, e.g., gibberelline A (O'Neill et al. 1986), salicylic acid (Yalpani et al. 1991), oligogalacturonides (Rigby et al. 1994) and glucosinates (Brudenell et al. 1999).

Based on their ability to easily move along the transport pathways of plants, systemic herbicides have been divided into phloem-mobile, xylem-mobile and ambimobile ones - the latter capable to penetrate into both phloem and xylem. Assignment to a particular compound class depends on physico-chemical parameters such as the dissociation constant (pK_a) and lipophilicity (K_{ow}). Phloem-mobile are herbicides with a pK_a <4, characteristic of strong and medium acids, and with a medium lipophilicity, 1og K_{ow} 1 to 2.5–3 Herbicides with a pK_a > 5, characteristic of weaker acids, and non-ionized compounds must be more polar to move well. Xylem-mobile are herbicides with a pK_a>7, i.e., a low degree of ionization, and a medium lipophilicity (log K_{ow} in the range 0–4). Ambimobile are weak acids (pK_a > 7) with a high hydrophily (log K_{ow} < 0). Highly

lipophilic herbicides (log K_{ow} > 4) are often non-sytemic regardless of their pK_a value, (because they can not be transported in xylem or phloem (contact herbicides; Bromilow et al. 1990).

The translocation of herbicides in the phloem depends on the synthesis and translocation of carbohydrates in plant tissues. In higher plants two mechanisms of assimilate translocation have been identified: through the symplast and through the apoplast. As long as there are sufficient plasmodesmatal connections, movement of sugars from the mesophyll cells to the sieve tubes may occur via the plasmodesmata. If plasmodesmata are absent between mesophyll cells and sieve tube elements, transport from the mesophyll to the sieve tubes can not take place via the plasmodesmata. In this case it involves the release of sucrose from the cytosol of the mesophyll cells to the cell walls, the apoplast. Cells located between the mesophyll cells and the sieve elements take up the sucrose from the apoplast against a concentration gradient via a sucrose-proton-co-transport carrier in the plasma membranes (transfer cells). The presence of the sucrose carrier explains both the selectivity for the exported sucrose and the high sugar concentration in the sieve tubes. After entering the plant, herbicides behave like sucrose (Devine, Hall 1990).

Results of studies on the translocation of six penethylamine derivatives differing in K_{ow} and pK_a indicated that strong bases with a pK_a of 9.5 and medium lipophilicity log K_{ow} ~ 2–3) were assimilated by roots and translocated to shoots to the highest extent. Assimilation decreased significantly with a decrease in pH up to 5.0. This can be explained by the fact that amine bases in acidic medium capture protons (are protonated), acquire charge, and hence acquire a lower permeability for membranes (Inoue et al. 1998).

The lipophilicity of organic xenobiotics greatly affects the assimilation by roots and subsequent transport in the xylem, as confirmed by several studies on the behavior of fungicides, herbicides and insecticides in soybeans , and the maximum concentration of each pesticide in the xylem sap is reached at a og K_{ow} ~ 3 (Sicbald et al. 1999).

However, despite its' lipophilic character, the fungicide morpholine is systemic. To explore this phenomenon, assimilation and transport of labelled morpholine fungicides, i.e., ^{14}C-dodemorphe and ^{14}C-tridemorphe, at different pHs have been studied. With this aim barley seedlings were exposed to herbicides by bathing its roots in solution of their two compounds. Absorption and translocation were very low at pH 5, but increased by a factor of 2 at pH 8. At pH 8 the more lipophilic fungicide tridemorphe accumulated to a large extent in roots and was translocated to a moderate extent to shoots. Dodemorphe accumulated to a small extent in roots, but was translocated through the epidermis into the xylem very effectively (Chamberlain et al. 1998).

3 - TRANSFORMATION OF ABSORBED ORGANIC CONTAMINANTS IN PLANTS

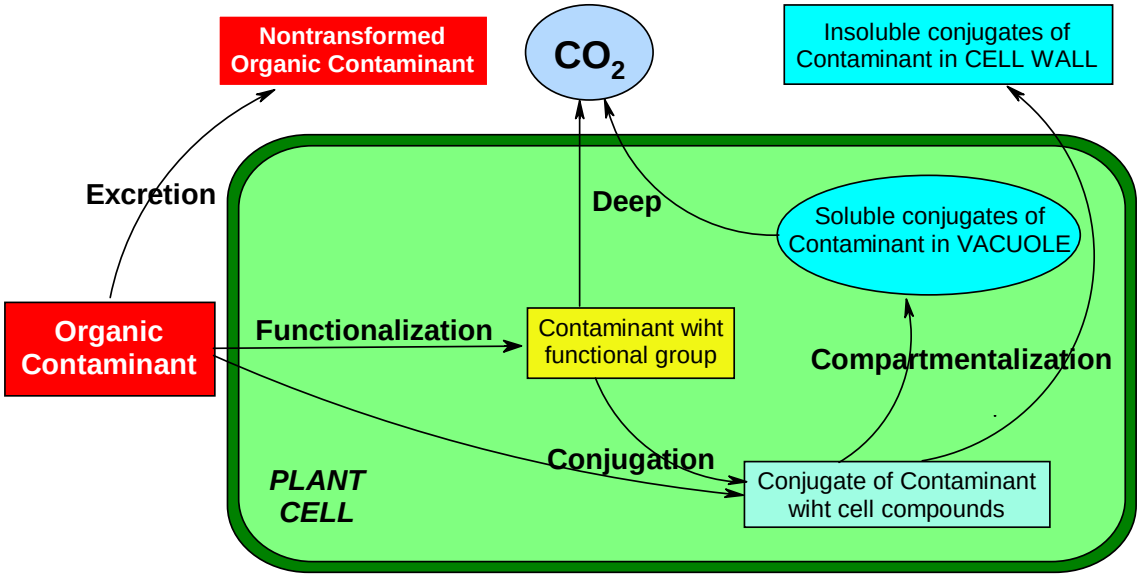

Figure 1. Major transformation pathways of organic contaminant transformation in plant cells.

Major transformation pathways of organic contaminants in plant cells are presented In Figure 1, and discussed below.

The most simple detoxification pathway is excretion, by which the contaminant molecule avoids (evades) cells being translocated through apoplast is excretred from the plant. This phenomenon is rare and occurs only at high concentrations of highly mobile (phloem-mobile or ambimobile) contaminants. More often xenobiotics penetrate the plant cells and are exposed to enzymatic transformations leading to the decrease of their toxicity. Recently, three phases in transformation of organic compounds were identified (Sandermann, 1994):

Phase I – Functionalization – when a molecule of a hydrophobic organic xenobiotic acquires a hydrophilic functional group (hydroxyl, amine, carboxyl) through enzymatic transformations (oxidation, reduction, hydrolysis, etc.). The polarity of the compound increases as a result of these processes and causes an increase in their affinity to enzymes catalyzing further transformation (conjugation or deep oxidation). In case of a low concentration, oxidative degradation of the compound to common metabolites of the cell and CO_2 takes place. Following this pathway a plant cell not only detoxifies the compound but also assimilates the resulting carbon atoms for cell needs. In case of a high concentration, full detoxification is not achieved and the contaminant is exposed to conjugation.

II Phase – Conjugation – when chemical coupling of the xenobiotic with cell compounds (proteins, peptides, amino acids, organic acids, mono-, oligo- and polysaccharides, pectins, lignin, etc.) occurs due to the formation of peptide, ether, ester and other bonds. Conjugated are intermediate products of xenobiotic transformation or the xenobiotics themselves when they already contain functional groups capable to react with the cell compounds. Conjugation increases hydrophilicity and movement of the xenobiotics. In a conjugated form the xenobiotic stays in the plant cell without interference with vital processes.

III Phase – Compartmentalization –a potential final step in the non-oxidative utilization of xenobiotics, by storage in certain compartments of the plant cell. Soluble conjugates (with peptides, sugars, amino acids etc.) are accumulated in vacuoles, insoluble conjugates (coupled with pectin, lignin, xylan and other polysaccaride) are taking out of the cell and accumulated in plant cell wall (Sandermann, 1987).

The three phases of transformation of xenobiotic compounds (functionalization-conjugation-compartmentalization) have been identified in chlorinated organic pesticides. For instance, the herbicide 2,4-D is conjugated with glucose and a malonyl residue by hydroxylation and is subsequently stored in the cell vacuoles (Sandermann, 1987).

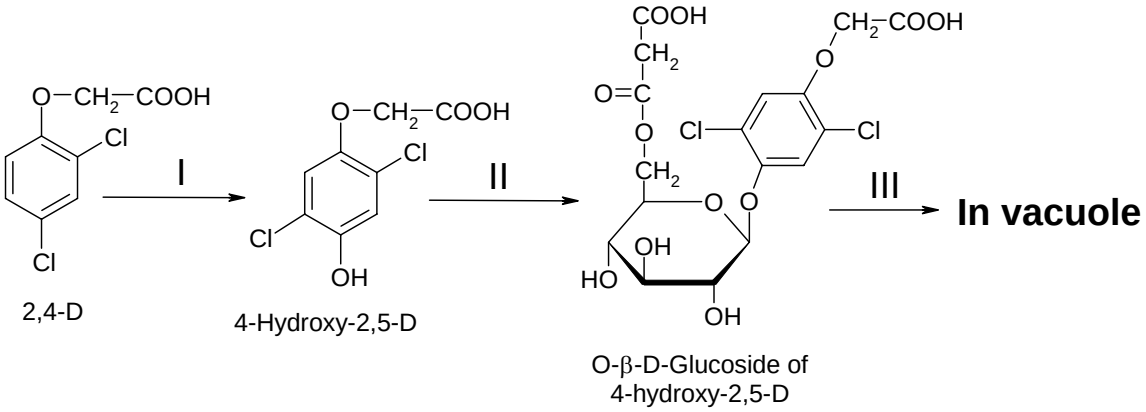

Figure 2. Pathway of 2,4-D transformation in plant cell

The insecticide DDT acquires a carboxyl group by primary oxidation, then turns into an ether with glucose using this carboxyl group and the formed conjugate is stored in the vacuole.

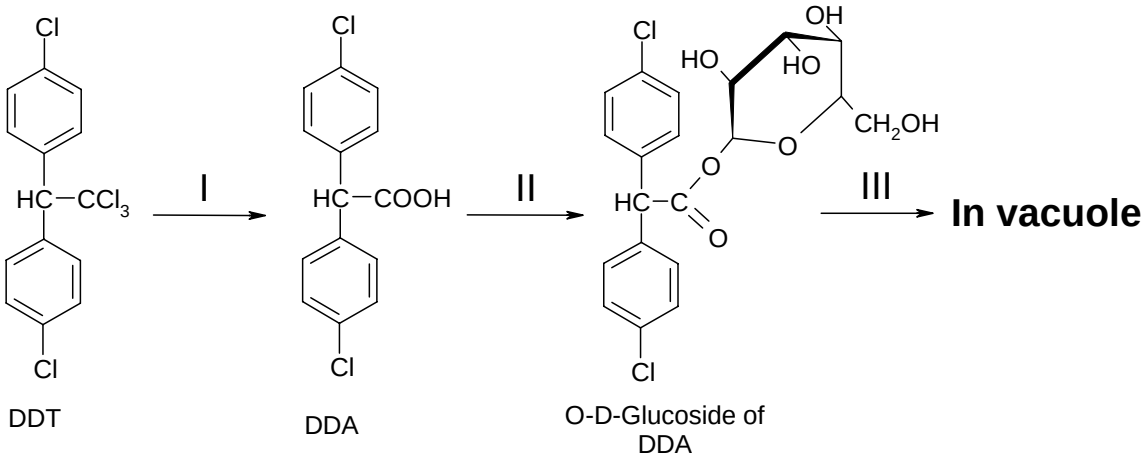

Figure 3. Pathway of DDT transformation in plant cell.

The biocide 2,3,4,5,6-pentachlorophenol is easily transformed into soluble β-D-glucoside and *o*-malonyl-β-D-glucoside conjugates, that have the tendency to move into vacuoles. However, when this toxicant acquires a second hydroxyl group during transformation, the resulting intermediate is conjugated with lignin and is stored in the cell wall.

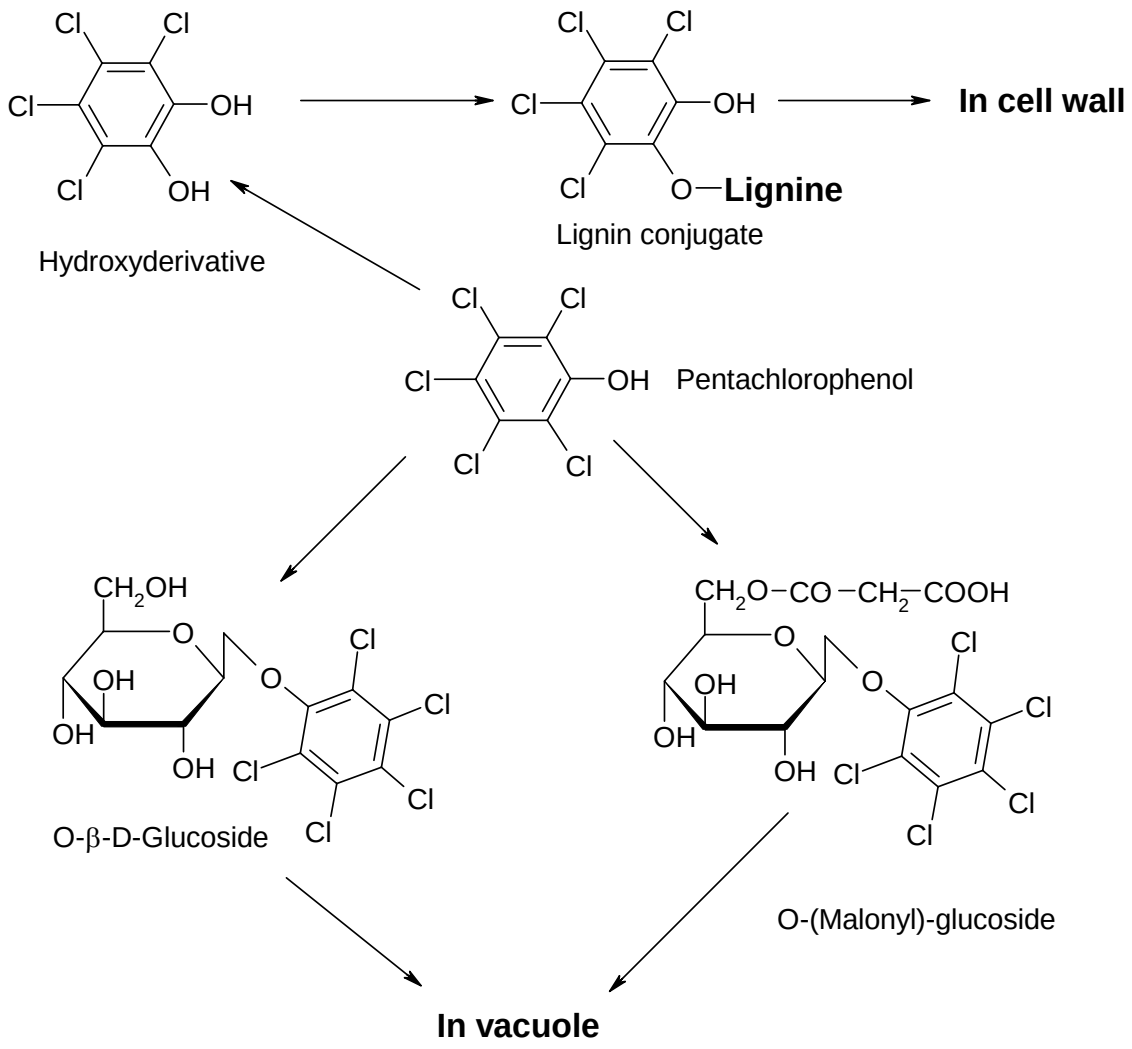

Figure 4. Pathway of 2,3,4,5,6-pentachlorophenol transformation in plant cell.

In the following sections the mechanisms of excretion, conjugation and degradation of xenobiotic compounds are discussed in more detail.

4 - EXCRETION

The term ‘excretion’ implies the partial release in unchanged form of a xenobiotic after absorption by a plant from leaves or roots (Korte et al., 2000; Zaalishvili et al., 2000). Xenobiotics are excreted via leaves after absorption by roots and vice versa. The principal differences between both processes are related to the entry of the compounds in the plant through either roots or leaves, and the translocation. Particularly, molecules of those compounds that are absorbed by roots and translocated along the apoplast, because they have a high mobility in the xylem (e.g. phenol) are excreted by the leaf stomata. Those compounds that are assimilated by leaves and translocated through the phloem, because they have a high mobility in the phloem or are ambimobile, are excreted by roots

in the soil or nutrient solution. One of the possible mechanisms of excretion of xenobiotics by roots is together with mucus.

The existence of these two excretion mechanisms is confirmed by many experimental data. Xenobiotic compounds, absorbed by leaves, and phloem-mobile are often excreted by roots. Such excretion is not always following the concentration gradient, it can also occur against the gradient. For instance, applied on leaves of soybean and wheat (*Triticum aestivum*) [^{14}C] alachlor is excreted via roots in the nutrient solution amended to a higher alachlor concentration than in the roots themselves (Chandler et al., 1974). Based on these data it was concluded that active transport is involved in the excretion by roots of phloem-mobile xenobiotics. The excretion of xenobiotics via the roots is the functional process, characteristic of higher plants.

Besides phloem-mobile, sometimes ambimobile xenobiotics absorbed by leaves are excreted via roots. Excretion by roots is characteristic for the phenoxyacetic acids (2,4-D, 2,4,5-T etc), dicambes, pocloram and other systemic herbicides (Hallmen, 1974; Long and Basler1974; Schultz and Burnside,1980; Lingle and Suttle, 1985). Roots are usually more active in the excretion of xenobiotics than leaves. For instance, roots of *Ampelampus albidus* excrete about 37% of the total amount of absorbed by plant leaves 2,4-D for 8 days (Dexter et al., 1971). Excretion of 2,4-D from roots is reported for other plants too: *Xanthium* sp.24% (for 4 days); *Ipomea purpurea* 21% (for 4 days); *Solanum rostratum* 15% (for 4 days); *Triticum vulgare* 11% (for 4 days); *Hordeum vulgare* 7% (for 8 days); *Avena sativa* 4% (for 8 days).

The closer the leaf-absorbing the xenobiotic is to roots the higher the rate of excretion by the roots is (Shultz and Burnside, 1988). The excretion rate also increases increasing herbicide concentration applied to the leaf. Excretion by roots occurs in herbidice-sensitive as well as -resistant plants (Dexter et al., 1971).

In the examples presented, most xenobiotics are excreted untransformed by the root system after absorption. The excreted amount is usually low, i.e., in the order of 0.1–2%. Nevertheless, the phenomenon of root excretion must be taken into account in cases where plants are treated with surficial herbicides, since it may become the source of serious chemical contamination of soil and groundwater.

Xenobiotics absorbed by roots are excreted by leaves. This phenomenon is far more rare than excretion by roots. For instance, phenol is excreted by the leaves of reed (*Scirpus lacustris*) after uptake by roots. Within 90 min. the air surrounding the leaves tests positive for phenol and within a couple of hours the phenol odor can be smelled (Seidel and Kickuth, 1965). Leaves of tobacco and radish absorbed 1,2-dibromomethane from solution through their petioles and subsequently excreted it through their leaves (Isaacson, 1986). Recent results of field experiments indicated, that poplar hybrids removed trichloroethylene (TCE) from artificially contaminated (260 mg L^{-1}) water and soil evaporated <10% of the total amount of TCE assimilated, and metabolized the rest (Kassel et al. 2002). Thus, plants can excrete halogen derivatives absorbed from soil, soil solution and groundwater.

5 - **CONJUGATE FORMATION**

Glycosylation of Hydroxylic Groups of Alcohols and Phenols

Glycosylation of xenobiotics is one of the main detoxification mechanisms of higher plants. Alcohols and phenols often undergo such transformations in plants.

This is illustrated by the formation of ethyl-β-glucoside in mung bean *(Phaseolus aureus)* seedlings cultivated in an ethanol-containing area (Middleton et al., 1978).

Other examples include:

- Glycosylation of geraniol after injection into apple *(Malus sylvestris)* into geranyl-β-D-glucoside (Wills and Scriven, 1979).
- Glycosylation of the alcohol hydroxyl group of saligenin into o-hydroxybenzyl-β-glucoside in broad bean seedlings (Pridham, 1958).
- Glycosylation of the herbicide *N*-hydroxymethyl dimethoate via a free primary alcohol hydroxyl group (Garner and Menzer, 1986).
- Glycosylation of pentachlorophenol in wheat and soybean plantsvia a combination with malonic acid, where. β-D-glucoside and o-malonyl-β-D-glucoside conjugates were simultaneously found in plant tissues (Schmitt et al., 1985).
- Glycosylation of xenobiotic mono-, di-, and triatomic phenols into corresponding β-monoglucosides in broad bean seedlings (Pridham, 1958).

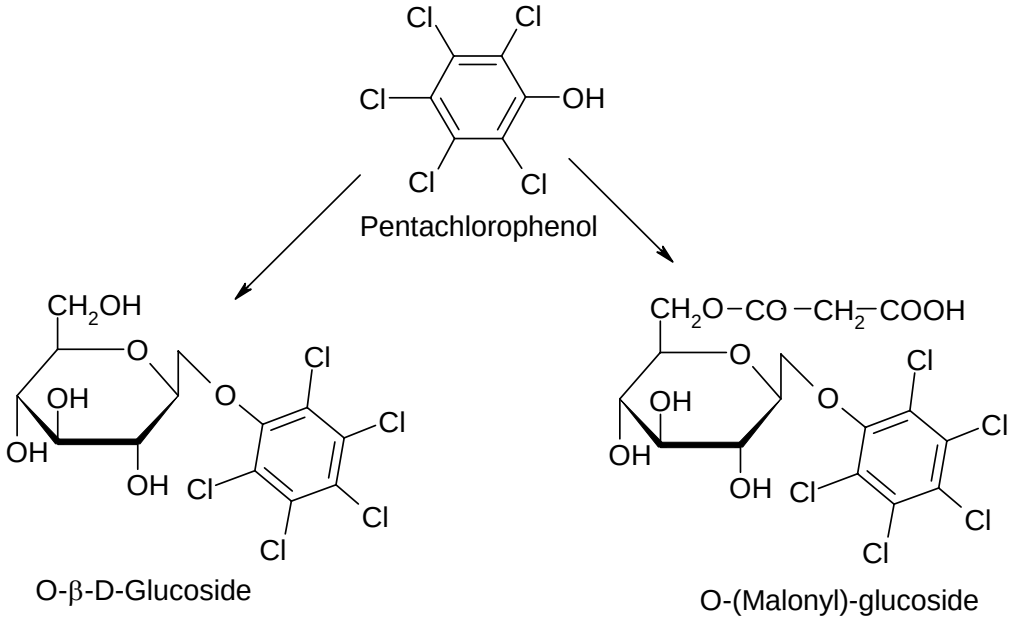

Figure 5. Conjugation of pentachlorophenol with glucose and malonyl-glucose.

In some cases where phenols are glycosylated di- and triglucosides are formed. For instance, diglucoside (gentiobioside) and triglucosides are formed from the xenobiotic hydroquinone in wheat *(Triticum rulgare)* embryos (Harborne, 1977).

Often, a hydroxy- derivative is formed as one of the primary products of transformation of xenobiotics in plant tissues, and is further subjected torapid glucosylation. Thus, a conjugate is formed of the oxidation product of the systemic fungicide etirimol (Harborne, 1977). The aliphatic side chain (butyl group) of this herbicide is oxidized and the alcoholic hydroxyl formed is glycosylated in leaves of barley *(Hordeum vulgare;* Harborne, 1977). The herbicide diphenamid is oxidized, i.e., the N-methyl group is hydroxylated, in pepper seedlings (Hodgson and Hoffer, 1977), and in callus tissue of tobacco after assimilation (Burrows and Leworthy, 1976).

Glycosylation of Carboxyl Groups of Organic Acids

Carboxyl groups of xenobiotic acids often undergo glycosylation in plants. For instance, the formation of esters with glucose is characteristic of phenoxyacetic acids. In root callus tissues of rice *(Oryza sativa)* in a liquid nutrient medium containing 2,4-[^{14}C]D, the glucosyl ester of 2,4-D was the main product isolated. However, no amino acid conjugates of 2,4-D identified in callus tissues of other plants were found. Thus, the main pathway of 2,4-D metabolism in rice root callus tissue is the formation of esters with glucose, but other pathways may operate in other plant species (Feung *et al.,* 1975).

2,4-D glucose esters occur widely and in large amounts in herbicide-resistant plants of wild wheat *(Triticum dicoccum),* timothy *(Phleum pratense),* and snapbean (Chkanikov *et al.,* 1976).

Besides carboxyl groups, other acidic groups are also subjected to glycosylation in plants. Thus, the plant growth regulator ethephon is glycosylated by formation of /?-D-glucopyranoside-l-(2-chloroethyl)-phosphonate in bark cuts of *Hevea brasiliensis* (Audley, 1979).

In some cases, other sugars than glucose also participate in the esterification reaction of the carboxyl group. For instance, arabinose esterifies with nicotinic acid in culture suspensions of parsley *(Petroselinum sativum)* (Leienbach *et al.,* 1975).

Glycosylation of Amino Groups

Glycosylation is a widespread way of blocking free amino groups of xenobiotics. Thus in 3-amino-2,5-dichlorobenzoic acid, is transformed further into an N-glucoside after transformation into its glucose ester in roots, shoots, and hypocotyls of *Setaria* sp. (Applied Environmental Microrbiology *et al.,* 1978).

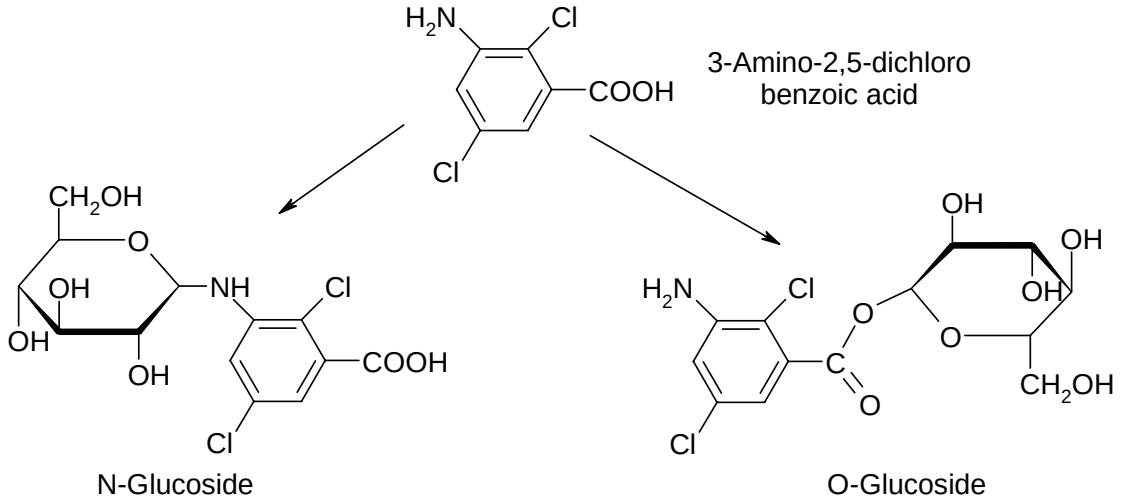

Figure 6. Conjugation of 3-amino-2,5-dichlorobenzoic acid with glucose by amino and carboxyl groups.

In studies on the glycosylation of synthetic cytokinin analogues in rootless seedlings of radish *(Raphanus sativus}*, it was found that the amides of 4-(purin-6-yl-amino)butanoic acid, 6,(34,-dimethoxybenzyl-amino)-purine, and 6-benzylaminopurine were converted into the corresponding 7-glucopyranosides, but adenine and methyl-aminopurine were not glycosylated under these conditions (Letham *et al.,* 1978). Ribosides were formed after the absorption of 6-benzylaminopurine in roots of snapbean seedlings (Ramina *et al.,* 1979). 4-chloroaniline and 3,4-dichloroaniline were glycosylated, and malonic conjugates were formed in wheat plants and culture suspensions of wheat and soybean (Wilkner and Sandermann, 1989). The herbicide metribuzin was first glycosylated and subsequently conjugated with malonic acid in tomato *(Lycopersicon esculentum)* ; Applied Environmental Microrbiology *et al.,* 1983b).

However, results of other studies on tomato biotypes with high, medium, and low sensitivity to metribuzin indicated, that the N-glycoside was the dominant metabolite (Smith *et al.,* 1989).

Conjugation of Carboxyl Groups with Amino Acids

Conjugation with amino acids is a widespread reaction of carboxyl groups of xenobiotics in plants. A study on 2,4-D metabolism in *Glycine* species demonstrated thatthe primary metabolite in resistant species was the glycoside conjugate of 4-oxy-2,4-D, but that in sensitive species conjugates with amino acids were formed (White *et al.*, 1990). 2,4-D formed conjugates with glutamic and aspartic acids in callus and differentiated root tissues of soybean (Davidonis *et al.,* 1978), in tissue cultures of maize endosperm, and in medullar parenchyma of tobacco, carrot, and sunflower (Feung *et al.*, 1975).

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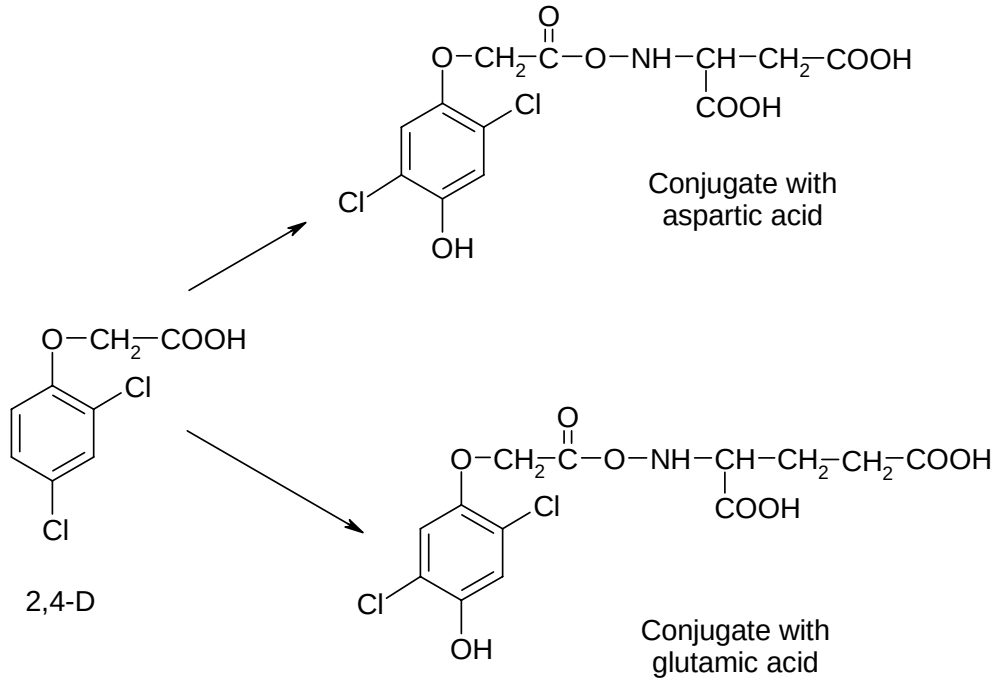


Figure 7. Conjugation of 24-D with amino acids.

Conjugation of Xenobiotics with Peptides

One of the most important detoxification abilities of higher plants is the conjugation of xenobiotics with the tripeptide glutathione. This detoxification pathway is most characteristic of symmetric triazines, chloroacetamides, and other halogen-containing compounds. A study on atrazine transformation in 53 herbaceous plant species *(Festucaeae, Avenae, Triticeae, Paniceae, Andropogenae, Eragrosteae, Chlorideae)* revealed that the herbicide formed conjugates with glutathione in all plants (Jensen *et al.*, 1977). An analysis of the atrazine transformation products formed in herbicide-resistant and -sensitive herbs revealed that in the resistant plants, big bluestem *(Andropogon qerardii vitman)* and switch-grass *(Panicum virgatum),* the major metabolite was the atrazine conjugate with glutathione. In the sensitive plants, Indian grass *(Sorghastrum nutans)* andsideoats grama *(Bouteloua curti pendula* Michx. Torr.), mainly N-deethylation products wereformed (Weimer *et al.*,1988). Studies on the metabolism of

atrazine in culture suspensions of wheat and potato indicated that the herbicide was transformed by N-deethylation in wheat cells, but by conjugation with glutathione in potato cells. The enzyme glutathione-S-transferase, capable of using atrazine as a substrate, was also identified in potato cells (Edwards and Owen, 1989). Conjugation with glutathione is characteristic of chloroacetamide herbicides (Le Baron *et al.,* 1988). For example, acetochlor forms conjugates with glutathione in seedlings of maize, morning-glory *(Ipomoea purpurea),* field bindweed *(Convolvulus arvensis),* cocklebur *(Xanthium pensylvanicum),* and velvetleaf *(Abutilon theophrasti)* (Breaux 1987). Applied to coleoptiles of maize seedlings, alachlor and metolachlor formed glutathione conjugates. The glutathione-S-transferase isolated from maize seedlings, which catalyzes xenobiotic conjugation with glutathione, had a three times higher activity when alachlor was used as a substrate than with metolachlor (O'Connel *et al.,* 1988). Active transformation of pretilachlor into its glutathione conjugate was observed in rice seedlings (Han and Hatzios, 1991). Compounds like benzyl chloride and propachlor form conjugates with glutathione by reactions catalyzed by enzymes and non-enzymatically (Han and Hatzios, 1991).

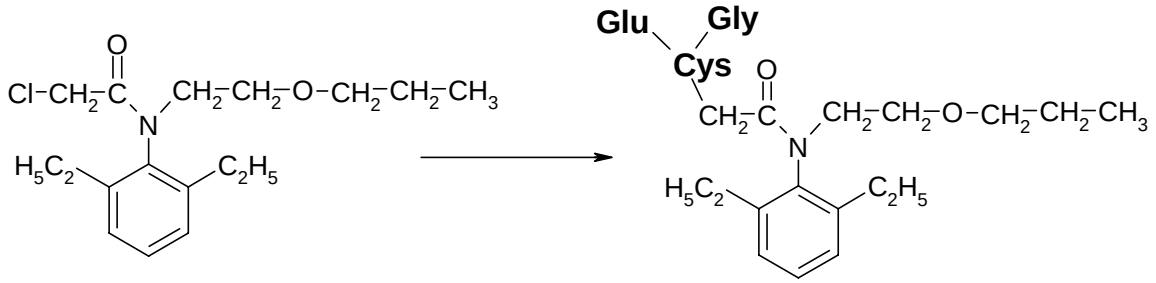


Figure 8. Conjugation of propachlor with reduced glutathione.

Another tripeptide, homogluthatione, may also participate in conjugation reactions with xenobiotics in plants. It differs from glutathione in containing P-alanine instead of glycine. Formation of homoglutathione conjugates is characteristic mainly of soybean. Thus, the herbicide propachlor forms a conjugate with homoglutathione in soybean seedlings (Lamoureux and Rusness, 1989). The same transformation occurs of chlorimuron-ethyl transformation (Brown and Neighborns,1987; Brown *et al.,* 1990). Homoglutathionic conjugates of acetochlor are formed also in other plants, particularly in soybean, mung bean, and alfalfa *(Medicago sativa)* (Breaux,1987).

Glutathione and homoglutathione conjugates of xenobiotics with a hydroxyl group are formed *in vivo.* Thus, acifluorofen, a derivative of diphenyl ether, is cleaved into 2-nitro-5-oxybenzoic acid in soybean seedlings, which couples to homoglutathione via its hydroxyl group (Applied Environmental Microrbiology *et al.*. 1983a).

Another mechanism characteristic for the binding of xenobiotics to glutathione and homoglutathione is the reaction with akyl-thio groups. The S-ethyldipropyi thiocarbamate conjugates with glutathione via an ethyl group in maize seedlings (Lay and

Casida, 1976; Carringer *et al.,* 1978). It is supposed that in this particular case the herbicide initially is oxidized into the corresponding sulfoxide an then is conjugated with glutathione. The latter process is catalyzed by glutathione-S-transferase. Metribuzin binds to homoglutathione via a methylthio group in soybean (Frear *et al.,* 1985). Benzo[a]pyrene is oxidized by conjugation with glutathione in microsomes from parsley *[Petroselmum hortense)* cell suspensions, soybean and primary leaves of pea seedlings (Trenck and Sandermann, 1980).

Phenol (oxybenzene) is not glycosylated in intact plants. A study of [l,6-^{14}C]phenol metabolism in sterile seedlings of maize, pea, and pumpkin (*Cucurbita pepo)* demonstrated that phenols form conjugates with low-molecular-weight peptides in plants (Chrikishvili *et al.,* 1977; Ugrekhelidze *et al.,* 1997). Other mono-atomic phenols also form conjugates with peptides in plants, i.e., z-naphthol in maize, pea, and pumpkin seedlings (Ugrekhelidze and Arziani, 1980; Ugrekhelidze *et al.,* 1983); o-nitrophenol in pea seedlings (Ugrekhelidze and Arziani, 1980; Ugrekhelidze *et al.,* 1983); 2,4-D in maize, pumpkin, and pea seedlings (Arziani *et al.,* 1983). Phenols are covalently bound to peptides via hydroxyl groups. The amino acid composition of peptides participating in the conjugation of phenols varies. In plants treated with phenol, the low-molecular-weight peptide concentration increases (Ugrekhelidze *et al.,* 1983). In some plants, conjugation with low-molecular-weight peptides seems to be an important detoxification pathway for mono-atomic phenols. Phenoxyacetic acids introduced into plant tissues form peptide conjugates. In sterile seedlings of maize and snap bean, phenoxyacetic and 2,4-dichlorophenoxyacetic acids form conjugates with low-molecular-weight peptides. In vine, the conjugates of these acids with peptides are formed (Mithaishvili *et al.,* 1979; Kakhniashvili *et al.,* 1979). As a result of hydrolysis of phenoxyacetic and 2,4-dichlorophenoxyacetic acids peptide conjugates, with 6 to 10 amino acids are formed (Kakhniashvili *et al.,* 1979; Kakhniashvili, 1988; Durmishidze *et al.,* 1982). In cereals, peptides/proteins participating in conjugation with phenoxyacetic acid contain from 2 to 220 amino acid residues (Chkanikov, 1985; Chkanikov *et al.,* 1982).

6 - DEGRADATION OF ORGANIC CONTAMINANTS

Hydroxylation

Introduction of a hydroxyl group into a xenobiotic molecule increases its polarity and hydrophilicity. In some cases, hydroxylation is the primary detoxification reaction, followed by the processes of profound oxidation and conjugation. A study on the metabolites of xenobiotic alkanes and N-alkali derivatives indicates that oxidative degradation of these molecules often starts with hydroxylation of alkyl groups. Although it is not always possible to isolate and identify the corresponding hydroxy derivatives, the products of their further metabolism provide information on the intermediates. Low-molecular-weight [C_l-$C_5{}^{14}$C]alkanes absorbed by leaves are subjected to oxidative transformation to $^{14}CO_2$ (Durmishidze and Ugrekhelidze, 1967; 1968a, b, 1975). Based on the identified intermediate products, it was concluded that these hydrocarbons are

oxidized monoterminally, with an intermediate formation of the corresponding primary alcohols, followed by oxidation to carbonic acids.

The hydroxylation of alkyl groups is a characteristic reaction in the transformation of urea-based herbicides in plants. In urea-based herbicides, the N-alkyl groups are subjected to hydroxylation. Fast oxidation of the hydroxylalkyl groups formed is accompanied by hydroxylation, generating a dealkylated product. N-dealkylation is the primary metabolic transformation pathway of *N*-methylphenyl urea herbicides. In some cases the hydroxylic groups formed are immediately glycosylated. Thus, the *β*-D-glucoside of the hydroxymethyl derivative of monuron was formed from [^{14}C]monuron in cotton leaves . Enzymatic cleavage or acidic hydrolysis of this glycosidic bond led to the formation of the corresponding demethylated products. Simultaneously, the formation of labeled formaldehyde was observed (Frear and Swanson, 1972). Analogous glycoside of an intermediate product of a durone hydroxymethyl derivative was isolated from sugar cane *(Saccharum officinarum)* (Liu *et al.*, 1978). Products of the hydroxylation of methyl groups (hydroxymethyl derivatives) are formed during the transformation of urea herbicides in plants: buturon in wheat (Hague *et al.,* 1977), monolinuron in spinach *(Spinacia oleracea)* (Schuphan and Ebmz, 1978), terbuthiuron in sugar cane (Loh *et al.*, 1978), chlorotoluron in wheat (Gross *et al.,* 1979). Chlorotoluron is hydroxylated in two positions: hydroxylation of the N-methyl group leads to demethylation, and hydroxylation of the methyl group bound to the aromatic ring leads to the formation of stable products (in contrast to the N-hydroxymethyl group, the C-hydroxymethyl group is stable; Gross *et al.,* 1979). Both products are formed in herbicide-resistant and -sensitive varieties of wheat (Cabanne *et al.,* 1985). However the two products are not formed in even amounts. The dominant metabolite is formed by N-demethylation (about 5.8%); the C-hydroxymethyl derivative presents as a minor component (about 1.4%). Analogously, by means of C-hydroxilation and N-demethylation is proceeded *1-sec*-butylphenol-N-methylcarbamate metabolization. Thin herbicide is absorbed by rice is subjected to hydroxylation by sec-butyl as well as by N-methyl groups (Ogawa *et al.,* 1976).

Sym-Triazines are subjected to N-dealkylation in plants. In the case of triazine herbicides, N-dealkylation proceeds by hydroxylation of a side chain (alkali group), but the corresponding hydroxy derivatives have not been identified. (Wichman and Byrnes, 1975; Pillai *et al.,* 1977; Weimer *et al.,*1988). However, despite the fact that the hydroxy derivative of atrazine was not found in culture suspension of potato and wheat, the product of hydroxylation of another sym-triazine, terbutryne was identified, and appeared to be the basic metabolite (Edwards and Owen, 1989). Moreover, it was found that [2^{14}C]terbacil, once absorbed by alfalfa, was hydroxylated via the methyl group (Rhodes, 1977).

Hydroxylation of the methylene group of xenobiotics has also been reported. Thus, carbofuran is hydroxylated at the C_3-atom in barley *(Hordeum vulgare*), maize (Penner and Early, 1973), and strawberry *(Fragaria vesca)* (Archer *et al.,* 1977).

The metabolism of [^{14}C]cyclohexane in plants indicates that the ring of this hydrocarbon is cleaved, and aliphatic products are formed. The first step in the

transformation of cyclohexane in plants is its hydroxylation into cyclohexanol (Ugrekhelidze, 1976).

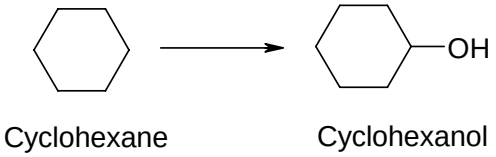


Figure 9. Hydroxilation of cyclohexane.

The first step in the metabolism of aromatic hydrocarbons in plants is the formation of hydroxy derivatives. [l,6-^{14}C]benzene is cleaved and aliphatic products are formed (muconic and fumaric acids) (Durmishidze et al., 1974a). The same products are formed from benzene in fruit (Durmishidze et al., 1974b).

Benzo[a]pyrene absorbed by plants is subjected to oxidative degradation and a significant portion of the carbon atoms are incorporated into the aliphatic compounds (Devdariani and Durmishidze, 1983, Devdariani, 1988). The analogous transformation of this xenobiotic was determined in cell culture suspensions (Harms, 1975; Harms *et al.*, 1977; Trenck and Sandermann, 1978). For such polycyclic hydrocarbons as naphthalene, benz[a] anthracene and dibenzanthracene, the same transformation pathways were observed (Devdariani and Kavtaradze, 1979; Devdariani *et al.*, 1979; Devdariani, 1988). Hydroxylation is believed to be the primary reaction in the transformation of polycyclic hydrocarbons in plants (Devdariani, 1988).

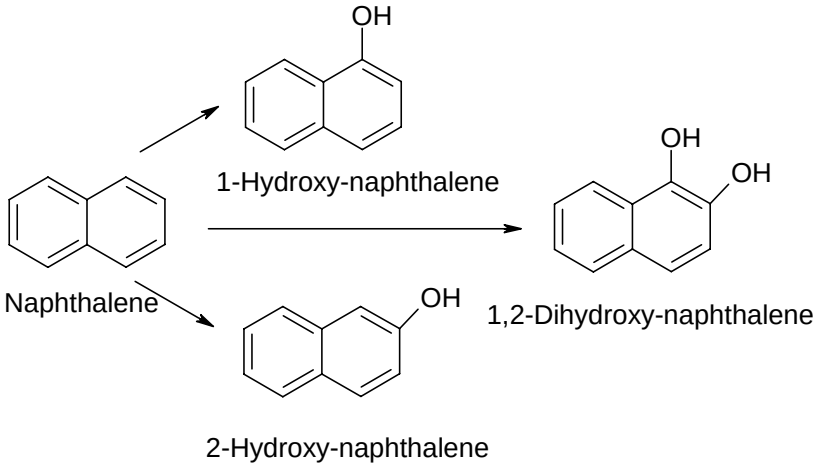


Figure 10. Hydroxilation of napthalene.

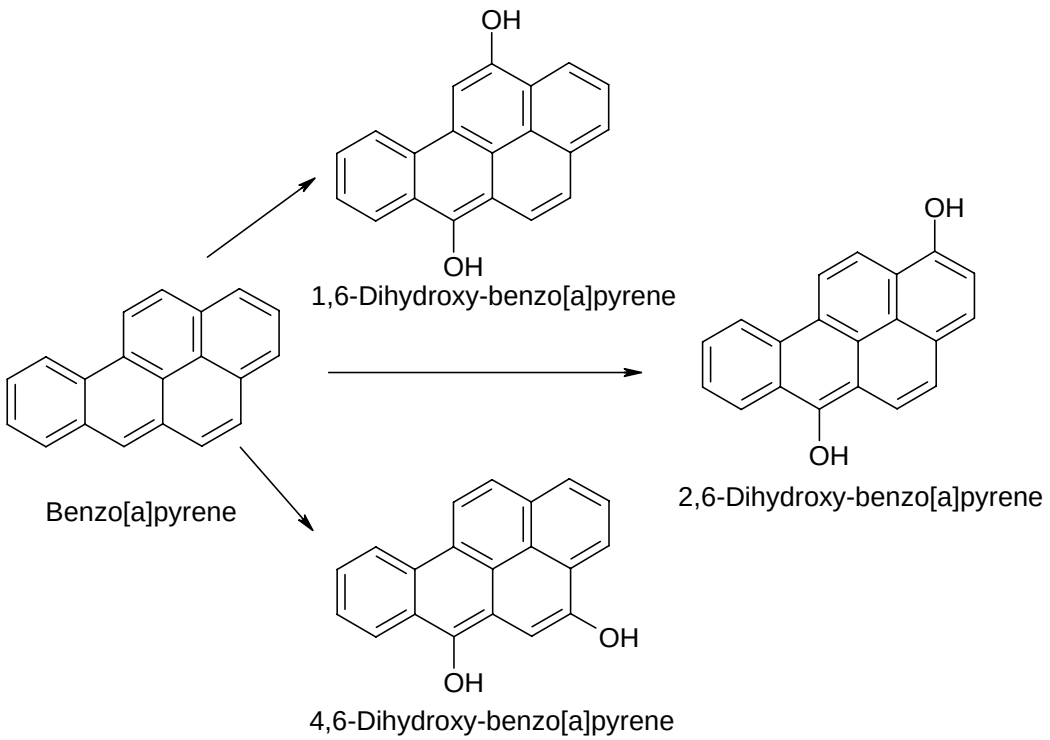


Figure 11. Hydroxilation of benzo[a]pyrene

Hydroxylation of the aromatic ring is an important step in the transformation of phenoxyacetic acid in plants. The introduced hydroxyl group is often subjected to glycosylation. Phenoxyacetic acid is hydroxylated mainly at position 4 of the aromatic ring. The hydroxylase activity increased 16-fold during the formation of the hydroxylated metabolite of phenoxyacetic acid (4-hydroxyphenoxyacetic acid) in oat *(Avena sativa)* seed embryos (Hutber *et al.*, 1978).

Phenoxyacetic acids halogenated in the aromatic ring are hydroxylated at non-substituted carbon atoms of the benzene ring. However, hydroxylation of 2,4-D often occurs at position 4 and a chlorine atom moves to positions 5 or 3. For example, identification of hydroxylated 2,4-D compounds in different plants such as wild buckwheat *(Polygonum convolvulus),* leafy spurge *(Euphorbia esula),* yellow foxtail *(Setaria glauca),* wild oat *(Avena fatua),* wild mustard *(Brassica caber),* perennial sowthistle *(Sonchnus arvensis),* and kochia *(Kochia scoparia)* has revealed that 2,5-dichloro-4-hydroxyphenoxyacetic acid is the dominant metabolite in all plants studied (Fleeker and Steen, 1971). A study on the transformation of 2,4-D in herbicide-sensitive and resistant *Glycine sp.* demonstrated that the 4-hydroxy derivative of 2,4-Dwas mostly formed in resistant species, and exclusively in the form of a glycoside (White *et al.*, 1990).

The herbicide diclofop (Shimabukuro *et al.,* 1987) and its methyl ether (diclofopmethyl) (Tanaka *et al.*,1990) are hydroxylated in plants in a similar way, although the product formed by glycosylation of the carboxyl group is the dominant

metabolite in some plants (Jacobson and Shimabukuro, 1984). The tolerance of certain ryegrass biotypes resistant and sensitive to diclofop does not depend on its metabolic products, since considerable amounts of the phytotoxic diclofop as well as its conjugates and hydroxylated ring derivatives are formed in stems and roots of both plant categories (Shimabukuro and Hoffer, 1991). The enzyme catalyzing the transformation of diclofop into 2-*β*-(2,5-dichloro-4-hydroxyphenoxy)phenoxy propionic acid was isolated and purified from etiolated wheat seedlings (McFadden *et al.,* 1989).

Benzoic acid and its derivatives are hydroxylated by various plants. Benzoic acid is hydroxylated simultaneously at o- and p-positions, and sometimes both hydroxy acids occur in tissues simultaneously. Dicamba is hydroxylated at position 5 and the product is the main herbicide metabolite in many plants (Chang and Vanden Born, 1971; Robocker and Zamora, 1976).

Isopropyi carbanilate and isopropyi m-chlorocarbanilate are also subjected to hydroxylation of the aromatic ring at different positions with subsequent conjugation of the hydroxy derivatives with glucose. Isopropyi carbanilate in alfalfa was transformed mainly into isopropyl-4-hydroxycarbanilate (Still and Mansager, 1975). Among wheat, sugar beet, and alfalfa plants, exposed to isopropyi carbanilate only wheat yielded isopropyl-4-hydroxycarbanilate, and additional 4-hydroxy and 2-hydroxy derivatives (Burt and Corbin, 1978). Isopropyl-3-chlorocarbanilate is hydroxylated into isopropyl-3-chloro-2-hydroxycarbanilate or isopropyl-3-chloro-4-hydroxycarbanilate (Still and Mansager, 1975).

The products resulting from the hydroxylation of aromatic rings are usually directly glycosylated at the hydroxyl group, and, therefore, isolation of the hydroxylation products is not always possible. However in definite cases identification of hydroxilated products is possible. For example, the herbicide bentazon is hydroxylated into 6-hydroxybentazon or 8-hydroxybentazon, followed by glycosylation, though in plant tissues treated with bentazon together with glucosides *the* initial herbicide hydroxy derivatives are also found (Connelly *et al.,* 1988; Leah *et al.,* 1989a,b).

Plants that are resistant to bentazon transform this herbicide rapidly, whereas sensitive plants transform this compound only slowly (Sterling and Blake, 1988, 1989, 1990).

The herbicide chlorosulfuron is hydroxylated at the aromatic ring in wheat seedlings and the hydroxy derivative undergo direct glycosylation (Sweetser *et al.,* 1982). However, the same herbicide is hydroxylated exclusively at the methyl group of the heterocyclic ring in seedlings of fiber flax *(Linum msitatissimum)* (Hutchinson *et al.*. 1984).

Herbicides of the sulfonylurea type are usually initially subjected to hydroxylation at the aromatic or heterocyclic ring or at the aliphatic radical, and the hydroxy derivatives are glycosylated (Beyer *et al.,* 1988). Thus, the sulfonylurea herbicide primisulfuron is hydroxylated at the pyrimidine ring, but hydroxylation of the benzene ring does not occur

in plants *(Echinochloa cross galli*; Neighbors and Privalle, 1990). On the other hand, microsomes from etiolated maize seedlings catalyze the hydroxylation of this herbicide at both the benzene and pyrimidine rings (Fonne-Pfister *et al.,* 1990).

In lettuce *(Lactuca sativa)* and vine, not only the aromatic ring and methyl group bound to it, but apparently also the methyl group of the N-methoxyacetyl radical of methalaxyl are hydroxylated simultaneously (Cole and Owen, 1987).

Finally, the rare hydroxylation at the amide nitrogen must be mentioned. The herbicide phenmedipham undergoes such hydroxylation in leaves of herbicide-resistant and -sensitive types of sugar beet, with transformation in resistant leaves being far higher than in sensitive leaves (Davies *et al.,* 1990).

Hydrolytic Cleavage

In most cases the ester bonds in xenobiotic molecules are cleaved. For example, 94% of the triclopyr esters absorbed by resistant wheat, tolerant barley, and sensitive common chickweed plants was hydrolyzed 3 days after treatment, and conjugated with glucose and aspartic acid (Lewer and Owen, 1990).

Three derivatives of sulfonylurea were metabolized with different rates in soybean plants indicating that cleavage rates of the ester bonds differed also (Brown and Neighbors, 1987; Brown *et al.,* 1990). Thifensulfuron-methyl was rapidly hydrolyzed into the corresponding thifensulfuronic acid, while the half-time of the thifensulfuric acid methyl ester in plant tissue being 4-6 h. Chlorimuron-ethyl, another ester, was de-esterified too, but more slowly, while conjugation with homoglutathione prevailed. Metsulfuron-methyl, the third ester, did not undergo de-esterification under the same conditions in the same soybean seedlings.

Xenobiotics with ester bonds are usually mainly transformed at the ester site. When ester bonds are lacking, other easily oxidized side groups of the diphenyl ether system are transformed and only in the latter are lacking cleavage of ether bonds occurs in wheat seedlings (Jacobson and Shimabukuro, 1984; Tanaka *et al.,* 1990), oats (Jacobson and Shimabukuro, 1984), oat culture suspension (Shimabukuro *et al.,* 1987), and ryegrass (Shimabukuro and Hoffer, 1991). Difenopenten-ethyl is de-esterified in soybean and wheat seedlings (Shimabukuro *et al.,* 1989). The highly selective diphenyl ether herbicide AKH-7088 is metabolized in soybean by complete oxidation of the side chain (Kouji *et al.,* 1990).

However, the acifluorofen molecule is cleaved at the ether bond in soybean, into the corresponding phenols that conjugate directly with glucose (and subsequently with malonic acid) and homoglutathione (Applied Environmental Microrbiology *et al.,* 1983). Similarly, fluorodifen (Eastin, 1971; Shutte and Golfman, 1975), nitrofen (Shutte and Golfman, 1975), and other diphenyl ethers are cleaved into the corresponding phenols. All these phenols are directly transformed into conjugates.

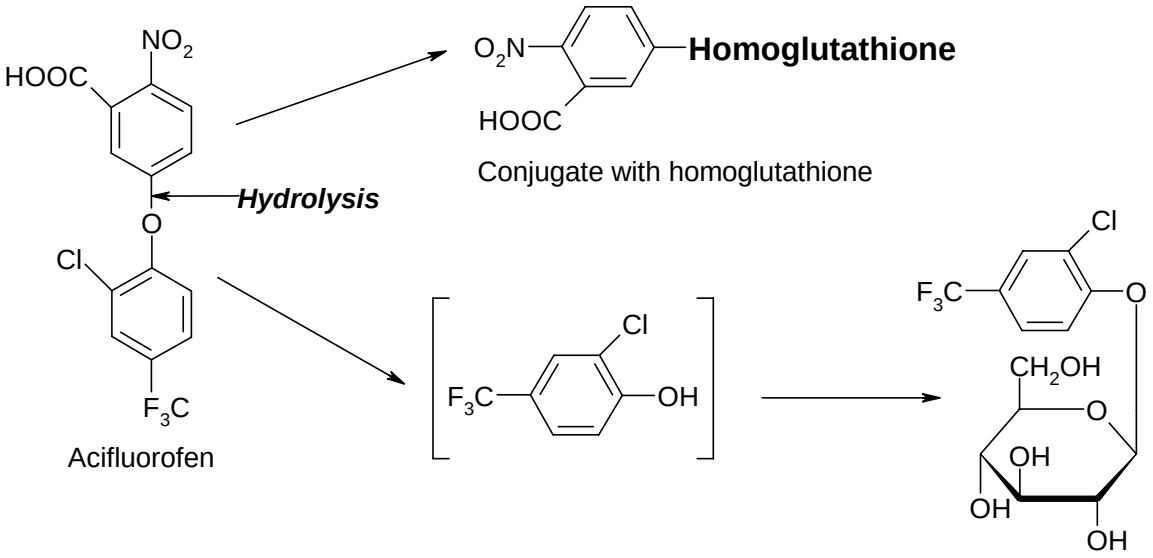


Figure 12. Hydrolysis and conjugation of acifluorofen.

Transformation of Explosives

2,4,6-Trinitrotoluene (TNT) is initially reduced to aminodinitrotoluenes (ADNTs) by most organisms. The tissue homogenates of rabbit livers, kidneys, or hearts were all capable to reduce TNT. Microorganisms of different taxonomic groups, i.e., fungi, bacteria, and yeast, were able to reduce TNT.

Under aerobic conditions, or conditions with limited oxygen availability, partially reduced nitrotoluenes and secondary condensation products are generated. These are completely reduced to TNT under strict anaerobic conditions. TNT and its reduced congeners converge during anaerobic treatment to tri-aminotoluene (TAT), which is chemically unstable (Rieger and Knackmuss, 1995).

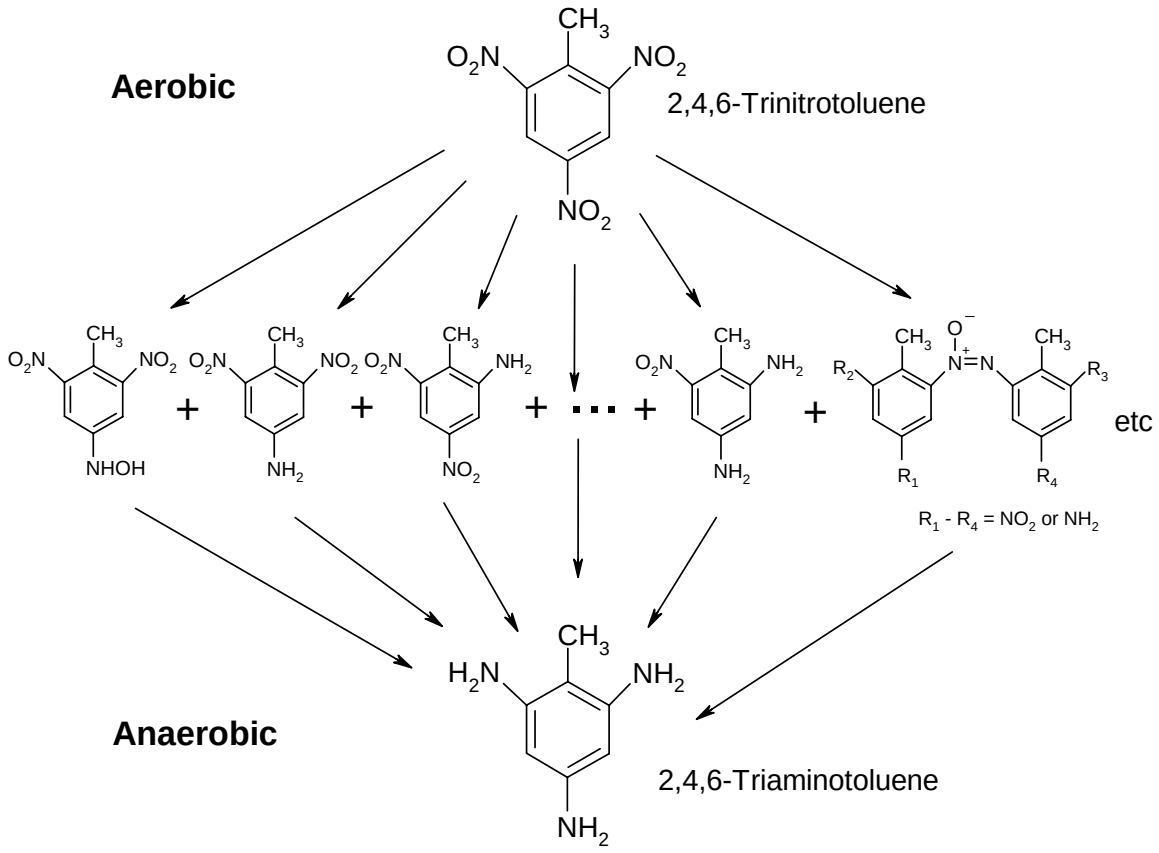


Figure 13. Reduction of 2,4,6-trinitrotoluene.

Some *Pseudomonas* strains and representatives of mycelial fungi are able to utilize TNT as a source of nitrogen and carbon, and incorporate atoms of these elements in the skeleton of their cell compounds. This is an example of how parts of xenobiotics can participate in the vital processes of organisms. *Phanerochaete chrysosporium* and some other basidial fungi completely mineralize TNT. Reduced metabolites of TNT are easily degraded by the enzymes of basidial cultures. Due to the high intra- and extracellular activities of oxidative enzymes, such as lignin peroxidase, Mn-peroxidase, and laccase the strains have a high degradational ability. These strains are the best microbial detoxifiers of various organic contaminants, including nitrogen-containing compounds.

The ability to absorb and assimilate TNT is also present in plants. The aquatic macrophyte parrot feather (*Myriophyllym aquaticum*) and the macroalgal stonewort (*Nitella*) are used for the remediation of TNT-contaminated water. The nitroreductase enzyme, which reduces the nitro groups of TNT, is active also in other algae, ferns, monocotyledonous and dicotyledonous plants. Tobacco plants have been successfully genetically engineered to express a bacterial nitroreductase gene, and acquired the ability to absorb and eliminate TNT from the soil of military proving grounds (Hannink et al., 2001).

Quite a few studies indicated that TNT 'disappears' from aqueous solutions in the presence of terrestrial or aquatic plants (Best et al., 1997; 1999a,b; Bhadra et al., 1999; Cataldo et al., 1989; Larson, et al., 1999). Hexahydro-1,3,5,-trinitro-1,3,5-triazine (RDX), another explosive, is absorbed by plants but its degradation is far slower than that of TNT. According to other studies, RDX is stable in solution and accumulates in plant tissues (Spain et al. 2000). The ability to take up and metabolize TNT in plants was confirmed by Hughes et al. (1997). They exposed three plant systems, i.e., *Catharanthus roseus* hairy root cultures, axenic and native *Myriophyllum* plants, to uniformly labelled ^{14}C-TNT, and evaluated the fate of ^{14}C. TNT was completely transformed in all plant systems containing viable plant tissue. They found the following metabolites: aminonitrotoluenes, unidentified ^{14}C-labelled compounds, extractable plant-associated ^{14}C fractions that could not be identified as reduction products and bound-residues (plant-associated material that could be quantified after combustion of the plant tissue).

TNT can also be reduced to 2,4,6-triaminotoulene (TAT) in plants (Rivera et al. 1998), and TAT subsequently undergoes ring cleavage. The enzymes that catalyse the reductions of the nitro groups of TNT are non-specific NAD(P)H-dependent nitroreductases (Esteve-Núñez, 2001). Complete reduction of the nitro groups significantly decreases the mutagenic potential of TNT.

Absorption and Enzymatic Transformation of TNT in Plants

Unpublished studies by Adamia et al. (2004); Khatisashvili et al. (2004); Gagelidze et al. (2004); Varsimashvili et al. (2004); Tinikashvili et al. (2004) on the absorption and enzymatic transformation of TNT in plants and microorganisms are briefly described in the following two sections. Plants were cultivated on running water under ambient illumination at 20-25^{o}C,, and 5-day seedlings were exposed to TNT in solution of 0.1 mM for 5 days. The following plant species were included in the tests: soybean (*Glycine max*), barley (*Hordeum sativum*), alfalfa (*Medicago sativa*), chickpea (*Cicer arietinum),* pea (*Pisum sativum*), ryegrass *(Lolium multiflorum),* sunflower (*Helianthus annuus*), and maize (*Zea mays*).

All plants greatly decreased the TNT concentration in the solution during 3-5 days (Fig.14). TNT disappearance was highest in the presence of soybean.

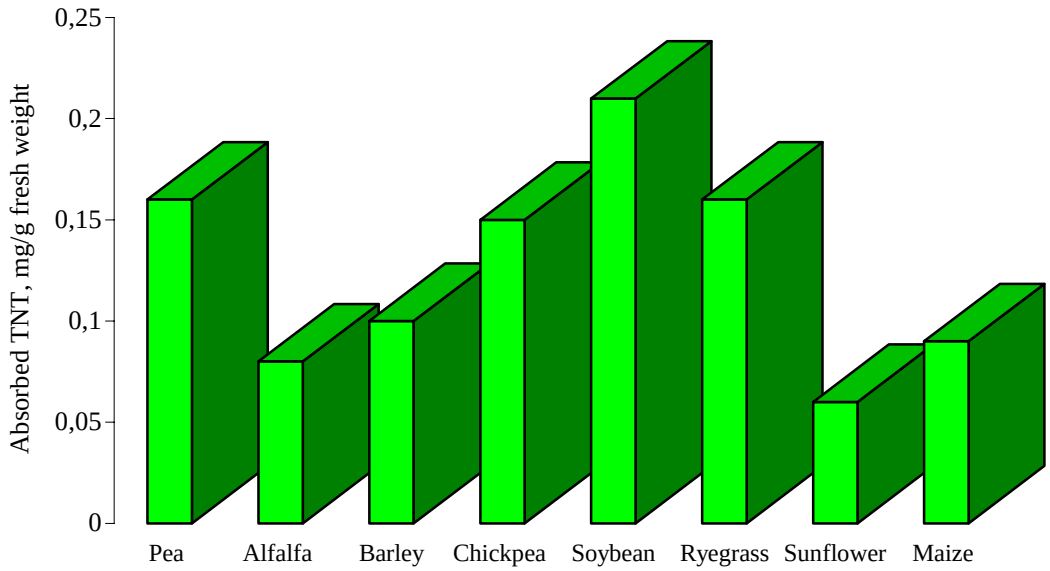


Fig.14. Disappearance of TNT in the presence of plants. The plants were cultivated in hydroponics. Five-day old seedlings were exposed to 0.1 mM TNT solution and the TNT concentration in the medium was measured after 3 days.

The distribution of [1-^{14}C]TNT in the cells of roots and leaves of plants was also studied using electron-microscopical autoradiography. TNT was mainly localized on membrane structures participating in the transportation of reductive equivalents (membranes of the endoplasmic reticulum, mitochondria, and plastids), in nuclei, nucleotides and vacuoles.

The TNT-derived ^{14}C in roots was mainly incorporated in low-molecular weight metabolites, but in stems and leaves in biopolymers (Fig.15). About 60% of the TNT-derived ^{14}C in soybean seedlings was bound to biopolymers of the upper plant portions.
The main metabolites of ^{14}C-TNT biodegradation have been identified using paper chromatography and autoradiography. Two groups of metabolites conjugated with biopolymers.

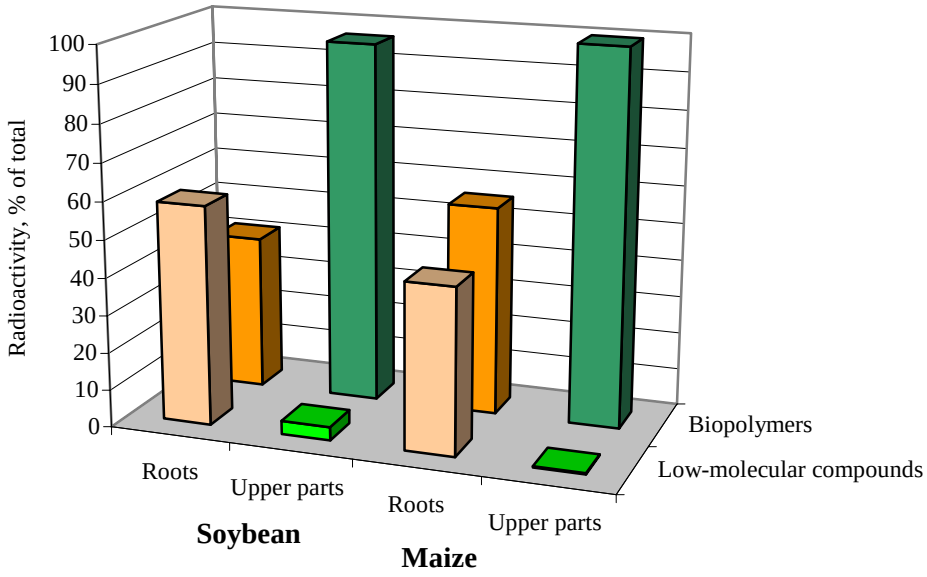


Figure 15. Uptake and distribution of radioactivity derived from (1-^{14}C) TNT between fractions of low- and high molecular compounds in plant roots and leaves. Specific radioactivity of TNT – 0.5KBq mg^{-1}, 0.5 mM solution in water, exposure – 5 days, natural illumination -25^{o}C).

About 80% of the metabolites contained amino groups and were reduction products of TNT nitro groups. Other compounds were formed by oxidation of the methyl group of TNT and contained a carboxyl group as a result. It turned out that most TNT metabolites in plants maintain the aromatic ring, and remain stable within the plant as they are chemically bound with biopolymer.

Enzymatic transformation of TNT was studied also in roots. Here degradation of TNT occurred largely by nitroreductase, that catalyzes the reduction of the nitro groups. Degradation was increased in the presence of the electron donors NADH and NADPH. Non-specific NAD(P)H-dependent nitroreductase activity was demonstrated in the cytosol in response to the exposure of the plants to the TNT in the medium. The increase of absorption of TNT from the solution was accompanied by the increase of nitroreductase activity in the plant (Fig.16). These results suggest that plant nitroreductase activity may serve as the biochemical criterion to select plants for the phytoremediation of soils contaminated with TNT.

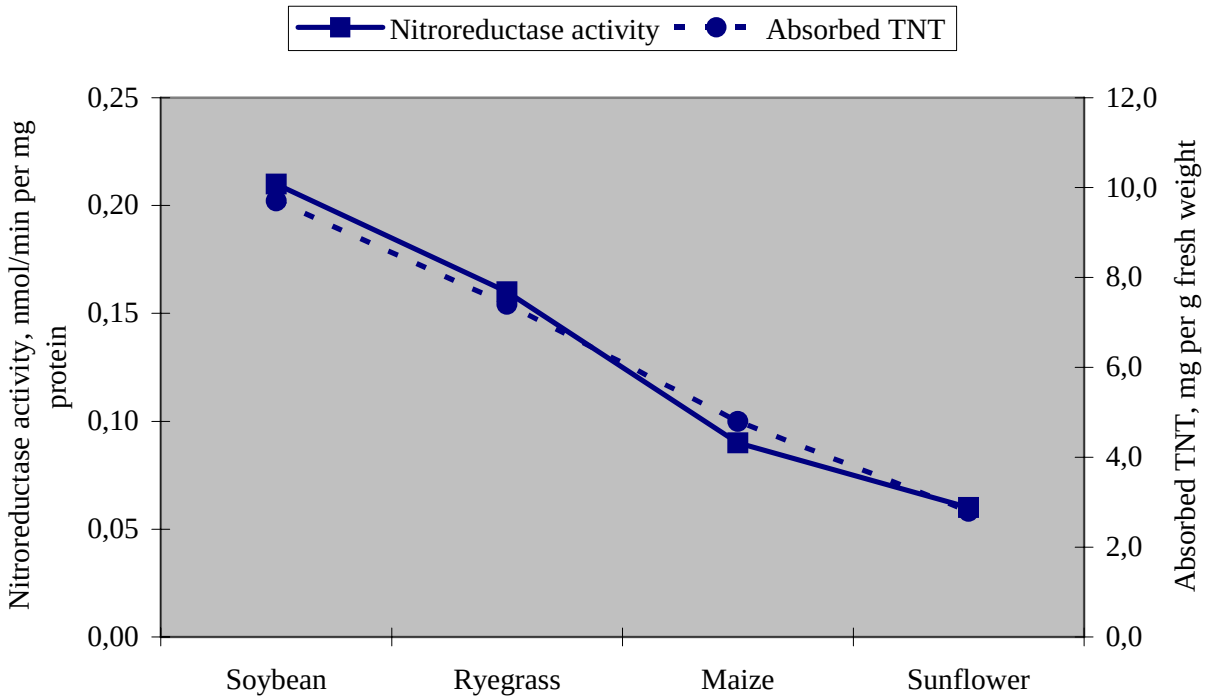


Figure 16. Correlation between quantity of absorbed TNT and nitroreductase activity of different plants.

The possibility that TNT is oxidized by oxidative enzymes in plants, such as cytochrome P450-containing monooxygenase, peroxidase and phenoloxidase, was investigated. The rationale of the experiment was that when oxidation of the methyl group of (C^3H_3)-TNT occurs, the tritium atoms are expected to be incorporated into water molecules and the radioactivity in the organic phase is expected to decrease. The following plant-based oxidizing enzymes were included in the tests: cytochrome P450-containing monooxygenase of soybean microsomes, horse-radish peroxidase and thea leaves phenoloxidase. Results indicated that the microsomal fraction oxidized [C^3H_3]TNT without any cofactors, but lost this ability in the presence of NADPH. It is probable, that cytochrome P450 is not capable of oxidizing [C^3H_3]TNT by itself under these conditions, without receiving electrons from the reductase. The other oxidative enzymes, phenoloxidase and peroxidase, effectively oxidized [C^3H_3]TNT. Based on these results it may be concluded, that biodegradation of TNT in plant cells can be accomplished by reduction as well as by oxidation. The latter process may involve the action of peroxidase and/or phenoloxidase. Since the activities and the inductibilities of the oxidative enzymes were far lower than in nitroreductase, it was concluded that the main pathway of TNT transformation in plants is a reduction of the nitro groups.

Transformation of TNT by Microorganisms

Autochthonic microorganisms were isolated from contaminated soils of military proving grounds. These soils contained typically 2.4 x10^4 to3.2 x10^7microbial cells per g

dry weight compared to 10-fold lower cell densities in control soils. Pure cultures of about 200 different strains were started, among which Rhodococcus spp. , mycobacteria and microscopic fungi (strains of *Aspergillus, Mucor, Trichoderma, Trichothecium*) prevailed. Some of these microbial strains have demonstrated the ability to degrade 80% of the TNT in soil with irrigated with an aqueous solution of 200 mg l^{-1} TNT (Table 1).

Table 1. Microbial capacity to assimilate TNT from sterile soil in 100 days (initial TNT concentration in soil– 200 mg kg^{-1}).

Microbial culture	Assimilated contaminant from soil, % initial contaminant concentration in soil	
	Red soil	Black soil
Bacteria similar to Nocardia sp. TNT-44	82	82
Aspergillus niger sp.35	82	80
Mucor sp.1	79	78
Rhodococcus sp.VKM Ac 1170 + TNT-44	82	73
Rhodococcus sp.VKM Ac 1170 + Aspergillus niger sp. 35	84	70

Low-molecular weight compounds have been identified, that were formed during the biodegradation of [1-^{14}C]-TNT by microorganisms with a high TNT-assimilation ability (Table 2). It was found that most of the radioactivity derived from ^{14}C-TNT transformation e ended up in organic acids and amino acids. Among the amino acids the TNT-derived radioactivity was recovered largely in the aromatic amino acids, and among the organic acids, largely in fumaric and succinic acids. Fumaric acid has also been identified as one of the products in the biodegradation of the benzene ring, and it is easily metabolized into succinic acid.

Table 2. Assimilation of [1-^{14}C]-TNT by microbes of different taxonomic groups. Specific activity of [1-^{14}C]-TNT – 500 Bq mg^{-1}; temperature - 30^{o}C; exposure time 5 days.
(x – unidentified acid).

Name or conventional number of culture	Radioactivity in biomass, % of total radioactivity applied	Radioactivity, % of total radioactivity recovered in the low-molecular compounds			
		Organic acids		Aminoacids	
Rhodococcus sp.VKM Ac 1170, str.44	51.2	70.7	Fumaric acid 67.4 x – 2.9 Glycolic acid – 0.4	29.3	Phenylalanine – 8.5 Glutamic acid – 5.4 Tyrosine – 5.1 Arginine – 3.0 Aspartic acid – 2.7 Serine – 2.1 Histidine – 1.8 Unidentified – 0.7
Mucor sp.1	52.7	100	Fumaric acid – 82.5 Succinic acid – 8.0 Unidentified – 5.8 Glycolic acid – 3.2 Malic acid – 1.2	0	
Aspergillus niger sp.35	52.9	100	Fumaric acid – 88.8 Unidentified – 5.3 Glycolic acid – 4.7 Malic acid – 1.2	0	
Saccharomy-ces oviformis	32.3	94.5	Unidentified – 59.1 Malic acid – 23.6 Succinic acid – 11.8	5.5	Tyrosine – 4.7 Aspartic acid – 0.8
Bacteria similar to Nocardia sp. TNT-44	24.1	98.8	Succinic acid – 54.2 Fumaric acid – 24.2 Glycolic acid – 10.7 Citric acid – 6.8 Unidentified – 2.9	1.2	Phenylalanine – 1.0 Tyrosine – 0.15 Arginine – 0.05
Bacteria similar to Nocardia sp. 136	42.5	94.6	Fumaric acid – 93.1 Succinic acid – 1.5	5.4	Phenylalanine – 1.8 Histidine – 1.6 Unidentified – 0.8 Tyrosine – 0.7 Aspartic acid – 0.4 Serine – 0.1

Based on the results presented in Table 2, it was concluded that the carbon skeleton of the TNT molecule undergoes deep degradation. In this process the initial step must be a reduction of the nitro groups, followed by use of the aromatic ring of the TNT molecule for the biosynthesis of aromatic amino acids. The subsequent oxidation leads to the removal of the amino groups and cleavage of aromatic ring, and generates organic acids . Organic acids are common cell metabolites. Thus, as a result of successive reduction and oxidation reactions complete detoxification of TNT occurs, and the atoms of this toxicant are included in the vital processes of the organism.

7 - DEEP OXIDATION

The above-presented data summarize the initial transformations of xenobiotics that penetrate the plant cell. The majority of low-molecular-weight substances formed after the transformation of the exogenous molecules accumulate in the vacuoles, just as secondary metabolites. Their further transformation is expected to proceed slowly, but this has not been confirmed experimentally.

In experiments on the absorption and transformation of xenobiotics with radioactively labeled carbon, the evolution of CO_2 is reported. Thus, deep oxidation of xenobiotics proceeds simultaneously with the initial transformations at which the basic structure –often composed by an aromatic ring- of the xenobiotic molecule is maintained (formation of conjugates).

Plants absorb alkanes and cycloalkanes from the environment and metabolize them. Experiments with ^{14}C-labeled hydrocarbons proved that sterile seedlings, placed in an atmosphere containing low-molecular-weight alkanes (C_1-C_5) or cyclohexane, absorb these compounds and transform them further by oxidation to the corresponding carbonic acids. Alkanes undergo monoterminal oxidation, while cyclohexane is oxidized via ring cleavage. The evolution of $^{14}CO_2$ in the dark during this process, serves as evidence for its' occurrence, and can be easily measured. Consequently, Organic acids and amino acids are end products of this degradation, which can be used further in cell metabolism (Ugrekhelidze, 1976). For instance, the transformation of methane in tea plant proceeds according to the following scheme:

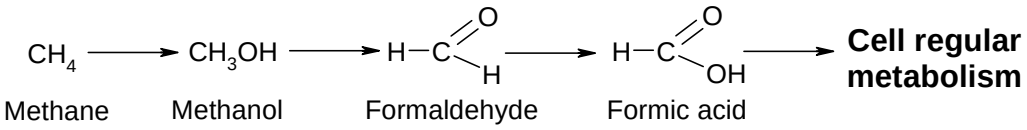


Figure 17. Scheme of methane degradation in plant cell.

Metabolism of ethane, propane and pentane leads to the formation of low molecular weight compounds largely composed by organic acids. Labeled fumaric, succinic, malonic, citric and lactic acid were identified in plant leaves, with most of the radioactivity incorporated into succinic and fumaric acid. Based on the fact that the carbon atoms originating from ethane were incorporated in these acids it is suggested that

ethane is oxidised monoterminally in plants. If ethane was oxidized at the two terminal carbon atoms, instead of one, the carbon atoms originating from ethane would be incorporated into glycolic, glyoxalic or oxalic acid. . The oxidation of ethane at one terminal carbon atom leads to the formation of acetyl-CoA, which in turn may participate in the Krebs cycle.

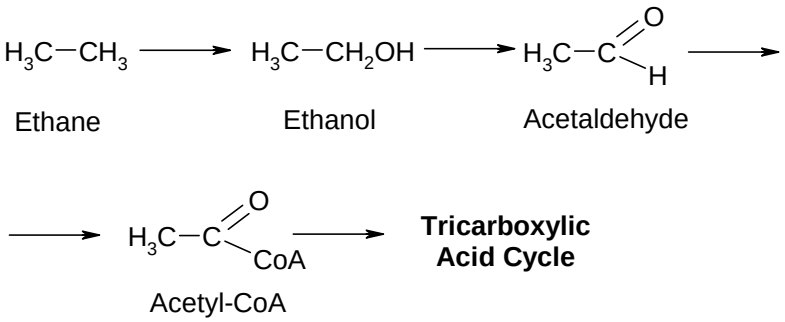


Figure 18. Scheme of ethane degradation in plant cell.

The oxidation of propane at one terminal carbon atom leads to the formation of propionic acid, which successively undergoes β-oxidation resulting in malonyl-CoA, and decarboxilation resulting in acetyl-CoA.

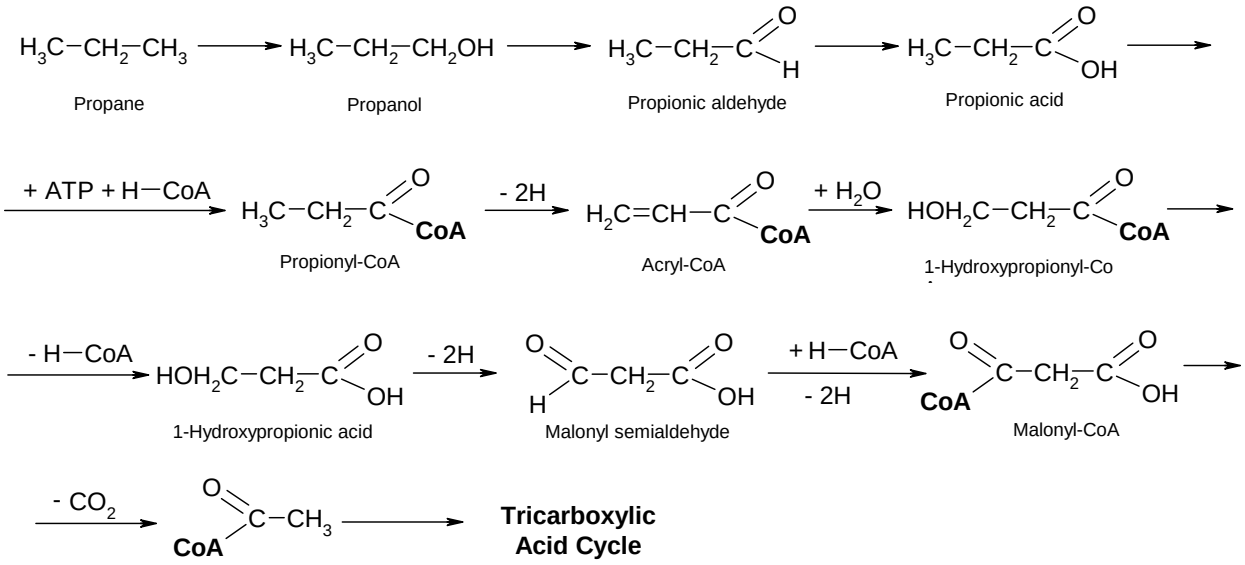


Figure 19. Scheme of propane degradation in plant cell.

Acetyl-CoA is transferred to the carboxylic groups of succinic acid. Based on the identified low-molecular degradation products it is suggested that propane is also oxidised monoterminally in plants into compounds that can be used in the Krebs cycle.Pentane may also be oxidized monoterminally, forming valeric acid. Approximately the same organic acids are formed from pentane as from valeric acid.

The abovementioned experiments may have been influenced by the phenomenon that the $^{14}CO_2$ evaporated during the oxidation of labeled ethane, propane and pentane was fixed again by the plants leading to increased incorporation of ^{14}C-label in the organic acids.

The long-chain alkanes are subjected to a transformation similar to that of short-chain alkanes. For instance, after 40 min of incubation of leek leaves with an emulsion of exogenous [^{14}C]octadecane in water, 9.6% of the total label was detected in esters, 6.4% in alcohols and 4% in organic acids (Cassagne and Lessire, 1975).

Following a similar experimental approach, it was demonstrated that plants are also able to metabolize benzene and phenol via aromatic ring cleavage (Durmishidze *et al.*, 1974c). In this process the carbon atoms from these compounds were incorporated into carbonic acids and amino acids. Similar data were obtained for toluene (Tkhelidze 1969; Jansen and Olson, 1969; DurMishidze *et al.*, 1974b), α-naphtol (Ugrekhelidze and Kavtaradze, 1970) and benzidine (Durmishidze *et al.*, 1979).

Oxidation of benzene and phenol by crude enzyme extracts of plants yielded muconic acid after ring cleavage, with pyrocatechin as an intermediate (Durmishidze *et al.*, 1969).

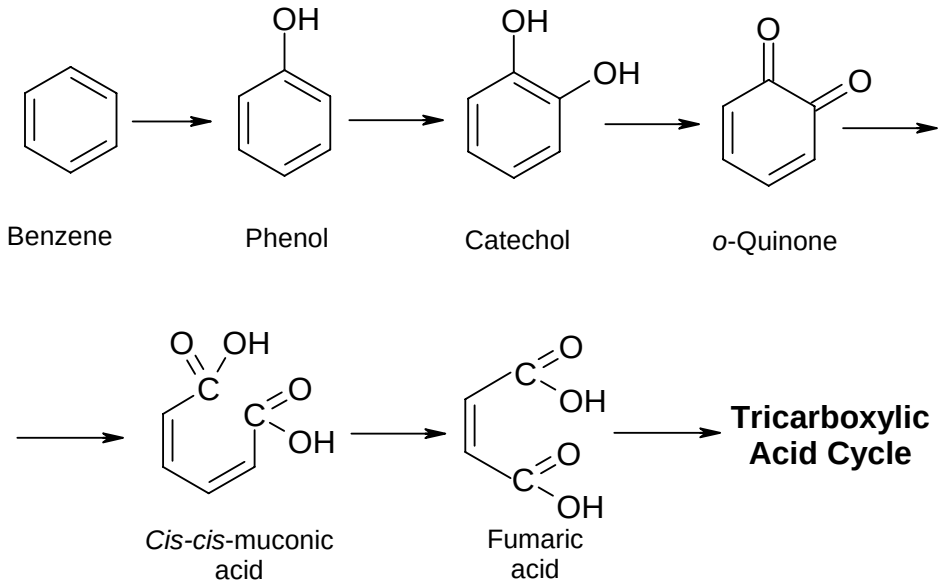


Figure 20. Scheme of benzene degradation in plant cell.

Further oxidation of muconic acid may lead to the formation of fumaric acid. Muconic acid and fumaric acid are often found in plants exposed to benzene or phenol. Cleavage of the aromatic ring in endogenous substrates proceeds the same way, i.e., 3,4-dihydroxybenzoic acid is transformed into 3-carboximuconic acid (Tateoka, 1970).

Phenoxyalkyl-carbonic acids with 4 and more carbon atoms in side chain often undergo *β*-oxidation in plants. For instance, 2,4-dichlorophenoxybutyric acid is oxidized

into 2,4-D (Hawf and Behrens, 1974; Taylor and Wain, 1978; McComb and McComb, 1978).

8 - ENZYMES PARTICIPATING IN THE DEGRADATION OF ORGANIC CONTAMINANTS

Reactions occurring during all three detoxification processes (functionalization, conjugation and compartmentalization) are enzymatic in nature. In the absence of xenobiotics these enzymes catalyze other reactions typical for regular plant cell metabolism. The following enzymes participate in the initial chemical modification of organic contaminants:

- oxidases, catalyzing hydroxylation, demethylation and other oxidative reactions – cytochrome P450 containing monooxygenases, peroxidases, phenoloxidases, ascorbatoxidase, catalase, etc.,
- reductases, catalyzing the reduction of nitro groups (nitroreductases),
- dehalogenases, splitting atoms of halogens from polyhalogenated toxicants,
- esterases, hydrolyzing ester bonds in pesticides and other toxic compounds.

Conjugation is catalyzed by transferases (glutathion-S-transferase, glucuronozyl-O-transferase etc). Compartmentalization of conjugates takes place with the participation of ATP-binding cassette (ABC) transporters (Eckardt, 2001). Depending on the structure of the xenobiotic other enzymes may also participate at different stages of the intracellular oxidation of the contaminant. For instance glutathion-S-conjugates that are deposited in the vacuoles are easily transformed into cystein conjugates catalyzed by peptidases (Coleman et al. 1997). During deep oxidation, enzymes involved in metabolism of secondary compounds, energetic and nitrogen metabolism participate also in the detoxification process by providing extra energy to the plant cell.

The main enzymatic reactions that provide functionalization of the organic contaminants in the plant cell are presented in the Figures below:

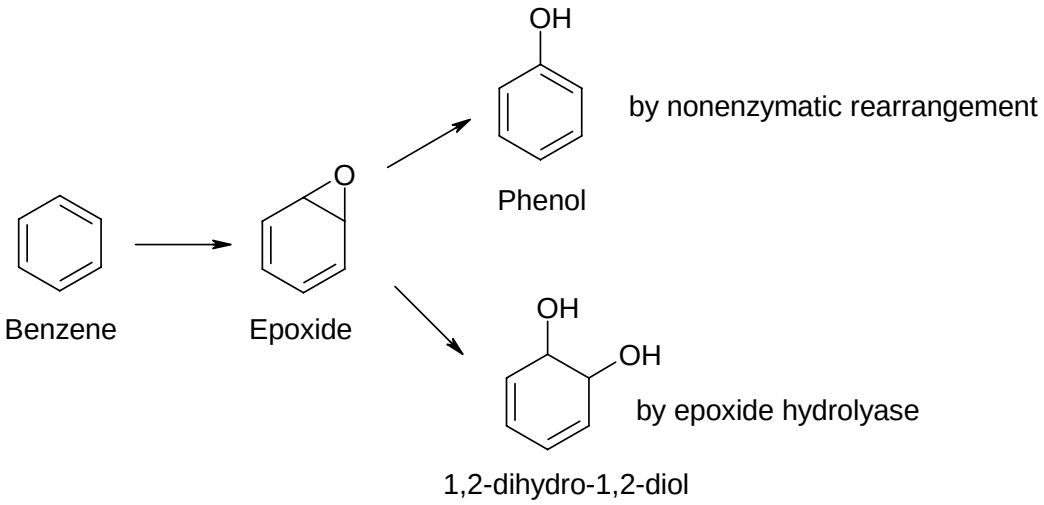


Figure 21. Aromatic hydroxylation (via epoxide).

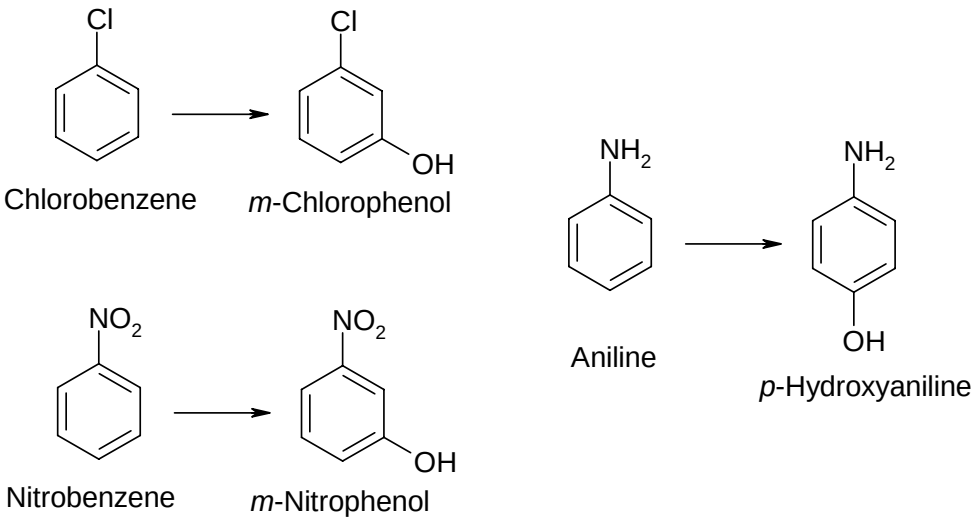
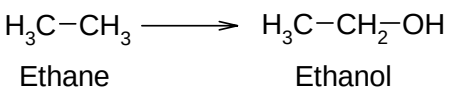


Figure 22. Aromatic hydroxylation (via O-insertion).



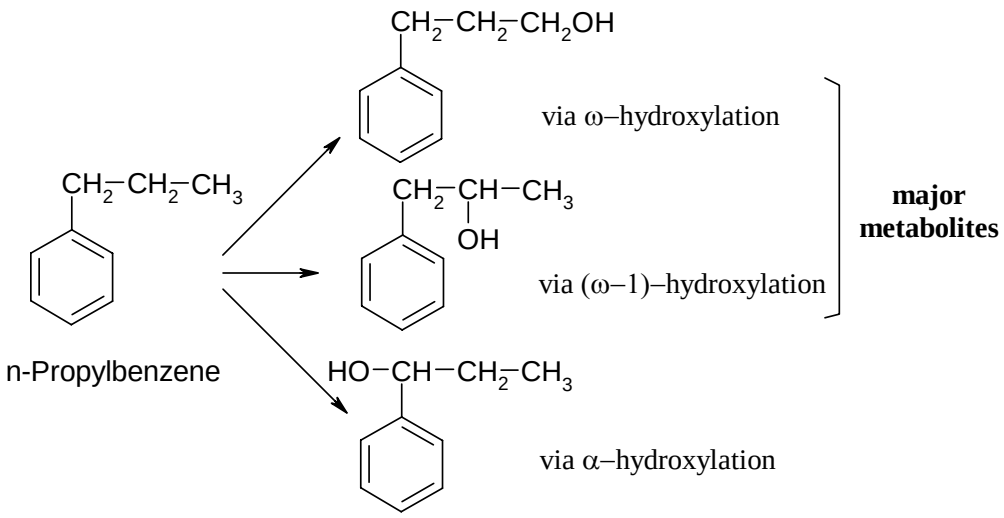


Figure 23. Aliphatic hydroxylation of n-propylbenzene.

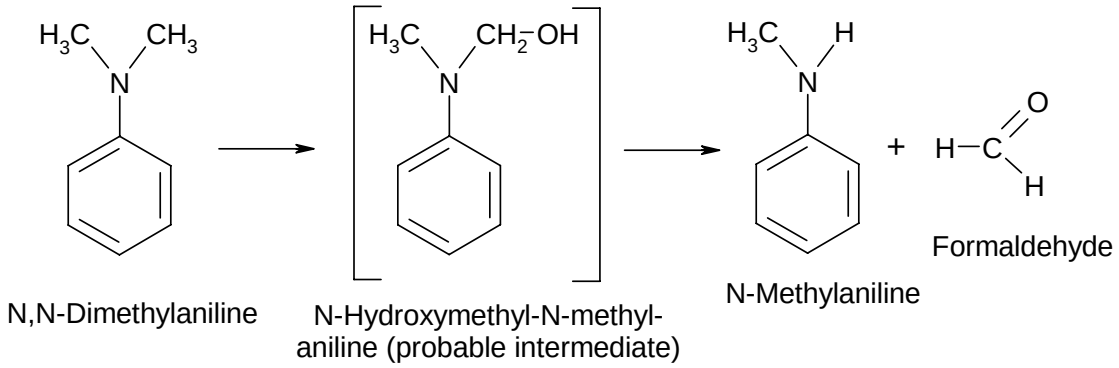


Figure 24. Dealkylation with oxidation of carbon atom of the alkyl group: N-dealkylation.

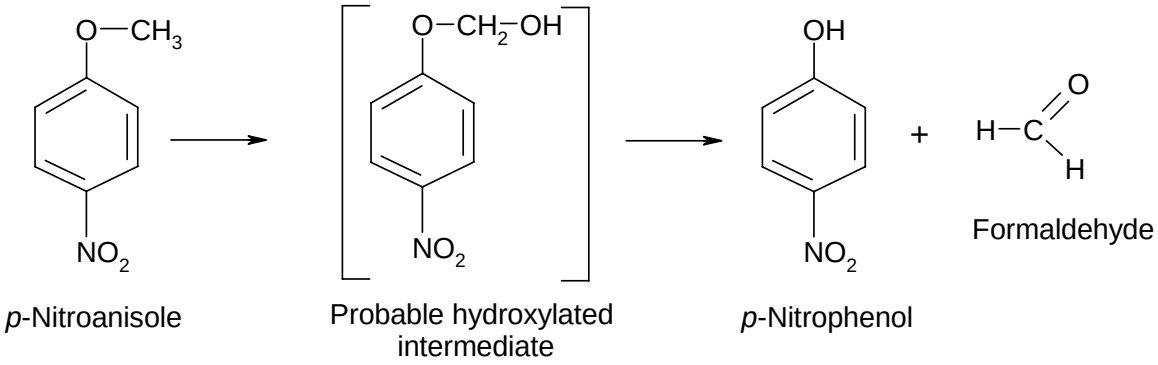


Figure 25. O-dealkylation of aromatic ether.

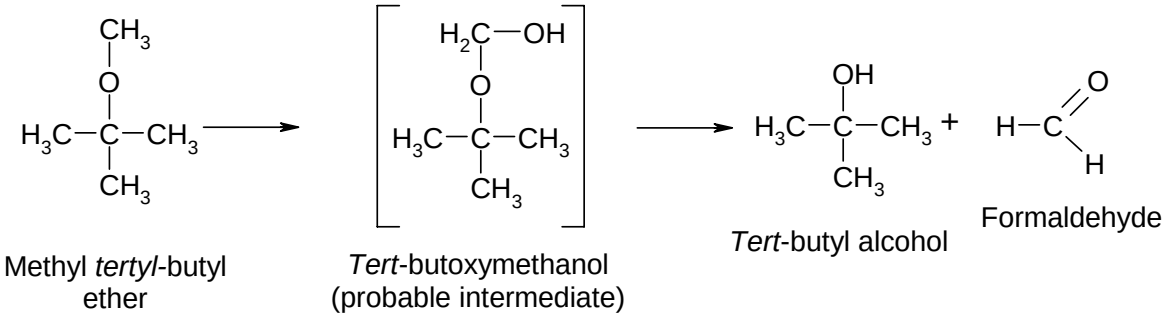


Figure 26. O-dealkylation of aliphatic ether.

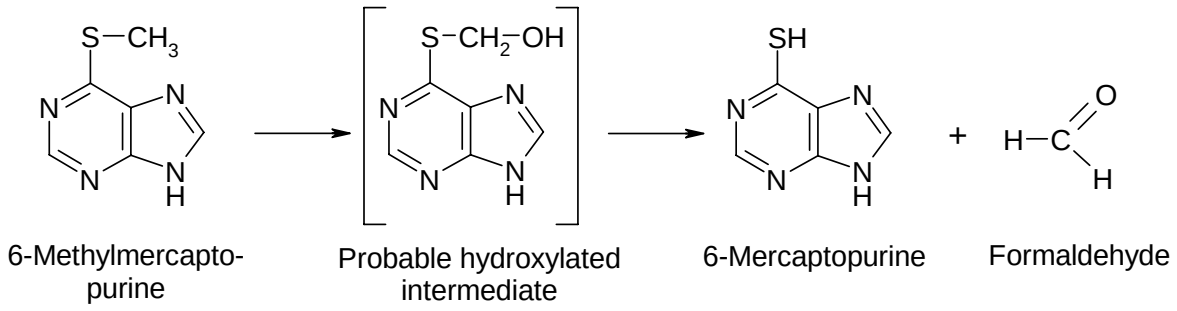


Figure 27. S-dealkylation of 6-methylmercaptopurine.

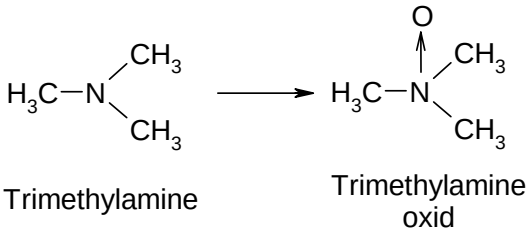


Figure 28. N-Oxidation (oxidation of nitrogen atom) of trimethylamine.

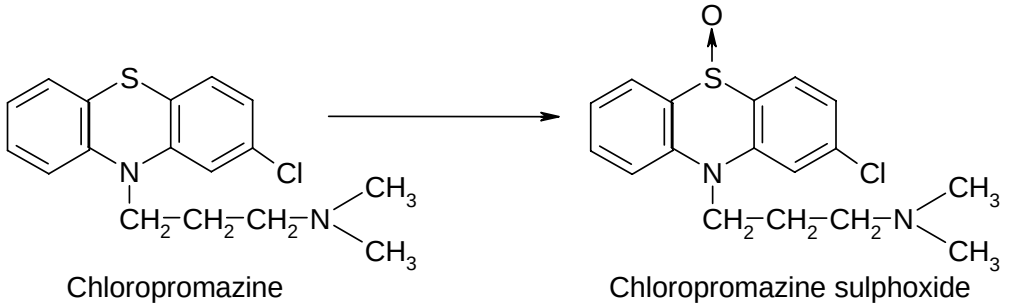


Figure 29. S-Oxidation (oxidation of sulfur atom) of chloropromazine.

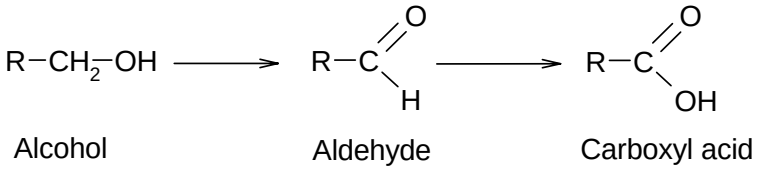


Figure 30. Alcohol and aldehyde oxidation

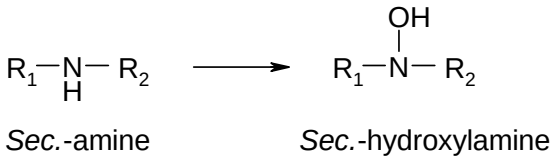


Figure 31. N-Hydroxylation of *sec*.-amines.

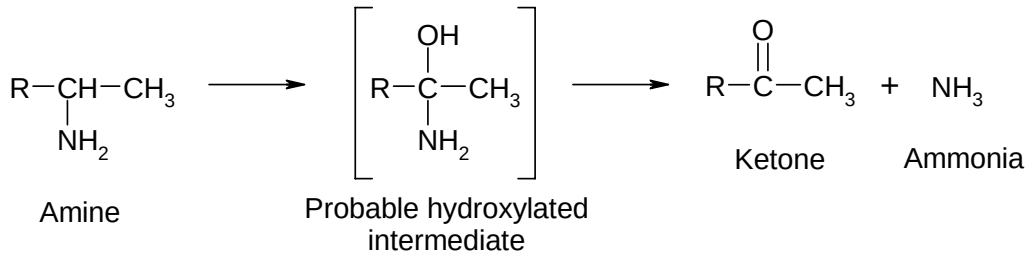


Figure 32. Oxidative deamination of *sec*.-amines

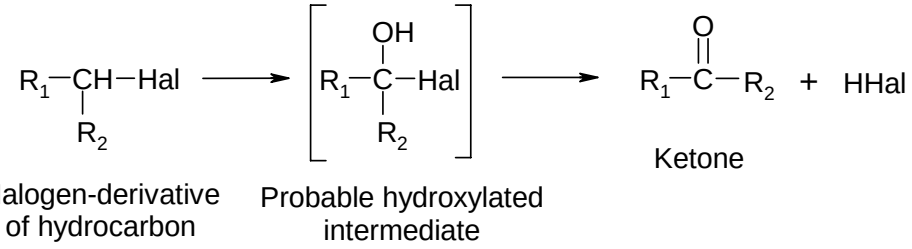


Figure 33. Oxidative dehalogenation of *sec*-halogenides.

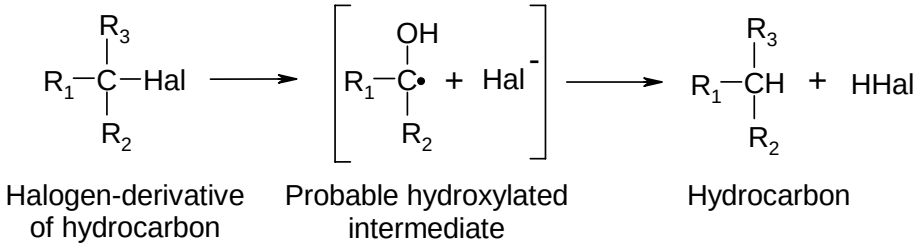


Figure 34. Reductive dehalogenation of *tert*-halogenides.

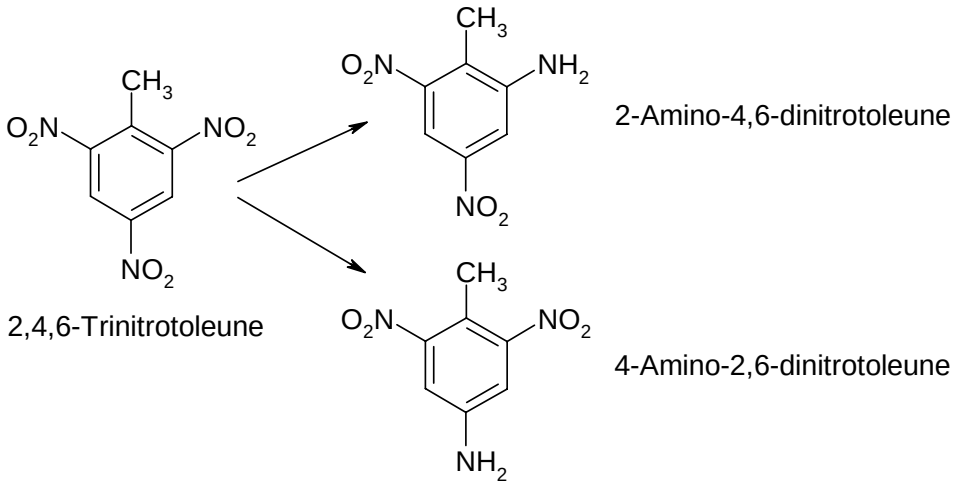


Figure 35. Nitroreduction of TNT.

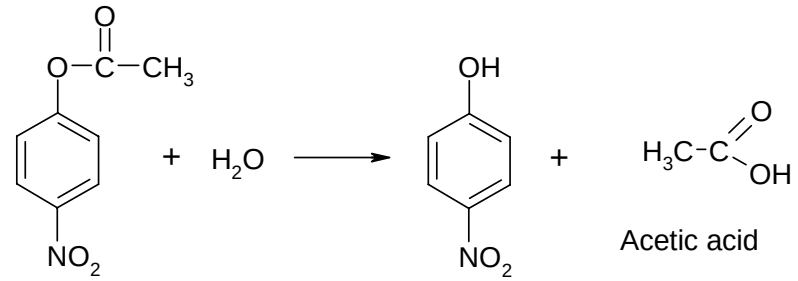


Figure 36. Hydrolysis of ester 4-nitrophenil acetate.

The characteristics of the key enzymes catalyzing the transformations mentioned in the Figures 21-36 are presented below.

Cytochrome P450-containig monooxygenases

Cytochrome P450-containig monooxygenases (EC 1.14.14.1) belong to one of the major classes of enzymes that are responsible for detoxification of organic contaminants in animals and plants (Robineau et al. 1998). They are mixed-function oxidases located in the membranes of the endoplasmic reticulum (microsomes), that utilize NADPH and/or NADH reductive equivalents for the activation of molecular oxygen and for the incorporation of one of its atoms into hydrophylic organic compounds (XH) that producefunctionalized products (XOH) (Schuler, 1996). In this case the second atom of oxygen is used for the formation of a water molecule.

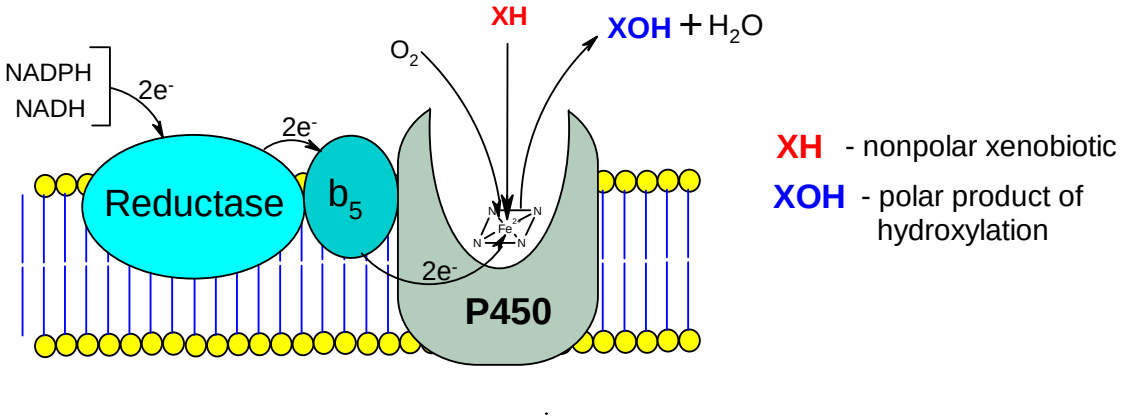


Figure 37. Microsomal monooxygenase system.

The microsomal cytochrome P450 containing monooxygenase system is the electron transfer chain, located in the membranes of the endoplasmic reticulum. This system contains the following components: the initial stage of electron transfer is a NADPH-cytochrome P450 reductase (EC 1.6.2.4); the intermediate carrier, cytochrome

b5; and the terminal acceptor of electrons, cytochrome P450. When NADPH is used as the only source of reductive equivalents in this system, the existence of an additional carrier, a NADH-dependent flavoprotein, becomes necessary. NADH may be oxidized also by the NADPH-dependent redox system. In the latter case $b_5$is not needed as the medium carrier (Hansikova *et al.*, 1994).

The cytochrome P450-dependent hydroxylation process consists of the following steps (Hodgson, Goldstein, 2001):

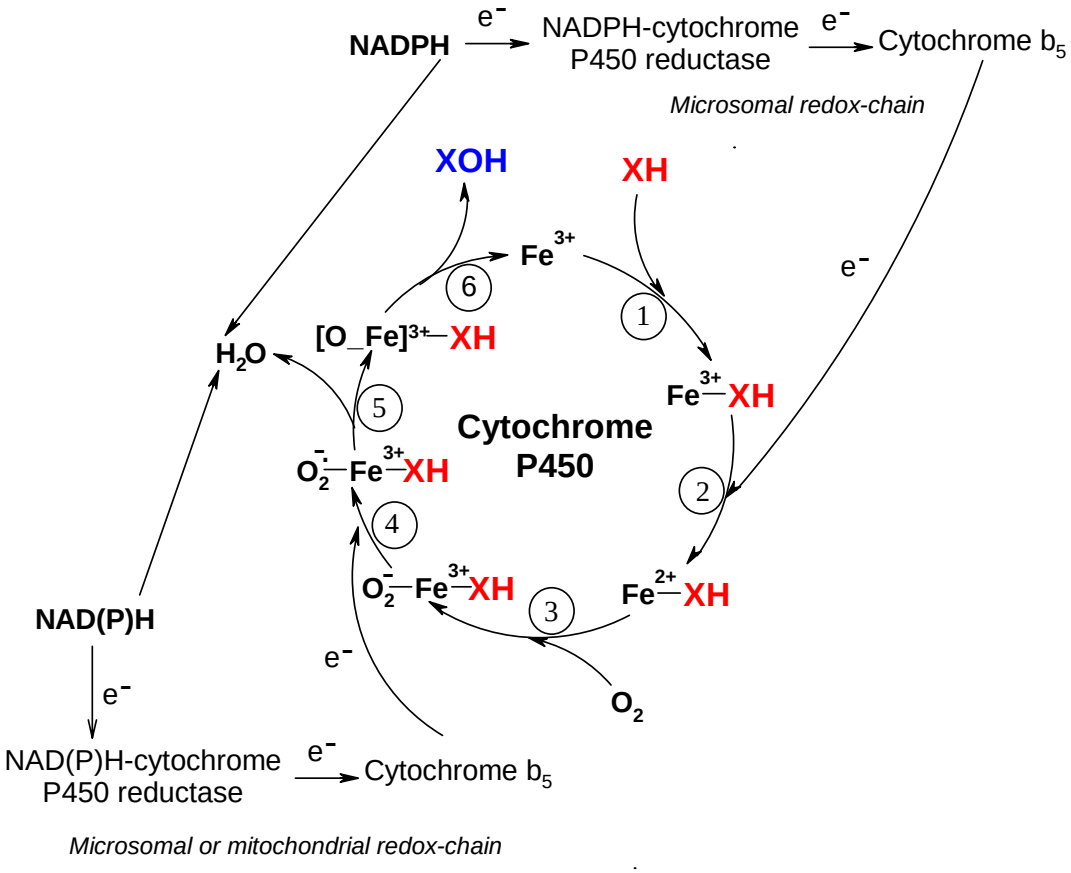


Figure 38. The mechanism of hydroxilation by cytochrome P450-containing monooxygenase.

1. The process begins when the contaminant (XH) binds tothe active site of the oxidized cytochrome P450 and an enzyme-substrate complex is formed, the so-called Ferricytochrome P450. If the reaction progresses further a water molecule is displaced that forms a ligand to the heme iron atom in the unbound P450. This is accompanied by a change in the spin of the Fe^{3+} from a low spin 1/2 state in which the $3d^5$ electrons are maximally paired, to a high spin 5/7 state in which the electrons are maximally unpaired. This in turn causes a change in the redox potential of the iron from approximately -300 mV to approximately -170 mV. This change is sufficient to render reducing the iron by the redox-partner of the cytochrome, usually NADPH or NADH, thermodynamically favourable.

2. The one-electron transfer from NADPH yields a complex of the reduced cytochrome P450 and the substrate, the so-called Ferrocytochrome P450. This reaction is catalyzed by the NADPH-cytochrome P450 reductase.
3. The reduced cytochrome P450-substrate complex reacts with molecular oxygen and forms the ternary complex, the so-called Oxycytochrome P450. This state is not stable and is easily autooxidised, releasing O_2. However, if the transfer of a second electron occurs (4), the catalytic reaction continues
4. The ternary complex undergoes a second one-electron reduction. This step is rate-limiting for the overall process of cytochrome P450-dependent hydroxylation. The reduction of the Oxycytochrome P450-substrate complex, the so-called Peroxycytochrome P450, takes place. The donor of the second one-electron transfer may vary with the substrate and/or availability of the reduced pyridine nucleotides. NADPH-cytochrome P450 reductase appeared predominant, but the electron maybe provided also from microsomal or mitochondrial NADH cytochrome b_5 reductase.
5. The oxygenated cytochrome P450 decomposes by releasing water. The O_2^{2-} then reacts with protons from the surrounding solvent to form H_2O that is released and leaves an activated oxygen atom.
6. The final step is the release of the hydroxylated substrate and the oxidized cytochrome P450: the oxidized cytochrome can then recycle by binding to another molecule of substrate.

This entire reaction cycle usually takes between 1 and 10 seconds.

Cytochrome P450-containing systems primarily fall into two major classes: bacterial/mitochondrial (type I), and microsomal (type II). Alternatively, cytochrome P450-containing systems can be classified according to the number of their protein components. Mitochondrial and most bacterial P450 systems have three components: an FAD-containing flavoprotein (NADPH or NADH-dependent reductase), an iron-sulfur protein, and cytochrome P450. The eukaryotic microsomal monooxygenase system contains two components: NADPH-cytochrome P450 reductase (a flavoprotein containing both FAD and FMN) and cytochrome P450. A soluble monooxygenase $P450_{BM-3}$ from *Bacillus megaterium* exists as a single polypeptide chain with two functional parts (the heme and flavin domains), and represents a unique bacterial one-component system. The sequence and functional comparisons show that these domains are more similar to cytochrome P450 and the flavoprotein of the microsomal two-component cytochrome P450 monooxygenase system, than to the relevant proteins of the three-component system.

The organization and functioning of the cytochrome P450-containing monooxygenase systems in procaryotic and eucaryotic organisms are distinguished by specialized features. Procaryotes contain soluble forms of this enzymatic system. In eucaryotes, the structure of the hemoproteins is established by their incorporation into the endoplasmic membrane (Archakov, 1983). The classic example is liver cytochrome P450, which is readily incorporated into the membrane structure. The individual components of the monooxygenase system are positioned along the entire membrane. In this configuration they are in close contact with the lipid matrix, which at the same time appears to have a barrier function. Oxidative hydroxylation in microsomes is, therefore,

preceded by the penetration of the xenobiotic through the membrane lipid layer. Formation of a catalytically active complex between cytochrome P450 and the xenobiotic determines its movement from the aqueous to the phospholipid phase.

Plants possess a cytochrome P450-containing monooxygenase system built into the membrane. However, plant cells also contain soluble forms of the same enzyme, enabling them to significantly enhance their detoxifying capacity (Gordeziani et al. 1991.

Cytochromes P450 are universally distributed and are present in animals, plants and microorganisms. Cytochromes P450 are encoded by a highly divergent gene superfamily containing over 450 cytochrome P450 (CYP) sequences distributed among 65 gene families, and exhibit a great diversity in reactive sites and amino acid composition. (Schuler, 1996). This superfamily contains a spectrum of CYP gene families that differ substantially in their primary sequence, substrate specificity, genomic organization and inducibility. Over 120 cDNA and genomic DNA sequences for P450s of different plants have been identified (Schuler, 1996): wheat (*Triticum aestivum),* avocado *(Percea anericana),* eggplant *(Solanum melongela cv.* Sinsadoharanasu), catmint (*Nepeta racemosa),* Madagaskar periwinkle (*Catharanthus roseus),* peppermint (*Mentha piperita),* pennycress *(Thlaspi arvense),* thale cress (*Arabidopsis thaliana),* maize *(Zea mays),* Jerusalem artichoke (*Helianthus tuberosus),* mung been (*Phaseolus aures),* alfalfa *(Medicago sativa),* sunflower (*Helianthus annuus),* pea (*Pisum sativum),* flaxseed (*Linum usitatissimum),* guayule *(Parthenium argentatum),* petunia *(Petunia hybrida),* moth orchid *(Phalaenopsis sp.* hybrid SM9108), sorghum (*Sorghum bicolor*), barberry (*Berberis stolonifera),* field mustard (*Brassica campestris),* pigeon pea (*Vicia faba),* tobacco (*Nicotiana tabacum),* soybean *(Glycine max),* etc.

Cytochromes P450 catalyze extremely diverse and often complex regio-specific and/or stereo-specific reactions in the biosynthesis or catabolism of plant bioactive molecules (Morant et al. 2003). It is possible to list more than 20 physiologically significant processes and reactions, in which cytochrome P450 plays a key role (Durst, 1991, Schuler, 1996). The most important of them are: biosynthesis of lignine monomers (Whetten, Sederoff, 1995), anthocyanins (Holton, Cornish, 1995), furanocoumarins (Berenbaum, Zangerl, 1996), gibberellins (Jenings et al. 1993), isoflavonoid phytoalexins (Kochs, Grisebach, 1986), alkaloids (Kutchan, 1995), hydroxylation of fatty acids (Salaün, Helvig, 1995), limonene and geranoil (Hallahan et al. 1994), etc. At the same time some plant cytochrome P450 containing monooxygenases can play an important role in hydroxylation of exogenous toxic compounds (pesticides, environmental pollutants and other xenobiotics) after they penetrate into the plant cell (Sandermann, 1994). Plant cytochromes P450 participate in the reactions of C- and N-hydroxylation of alyphatic and aromatic compounds, N-, O-, and S-dealkylation, sulpho-oxidation, deamination, N-oxidation, oxidative and reductive dehalogenation, etc. (Schuler, 1996). The biochemical resistance to many herbicides in plants is mediated by the rapid transformation of the herbicide into a hydroxylated, inactive product that is subsequently conjugated to carbohydrate moieties in the plant cell wall (Schuler, 1996). For examples, N-demethylation and ring-methyl hydroxylation of the phenylurea herbicide chlortoluron

in wheat and maize are cytochrome P450-dependent processes (Mougin et al. 1990; Fonné-Pfister, Kreuz, 1990):

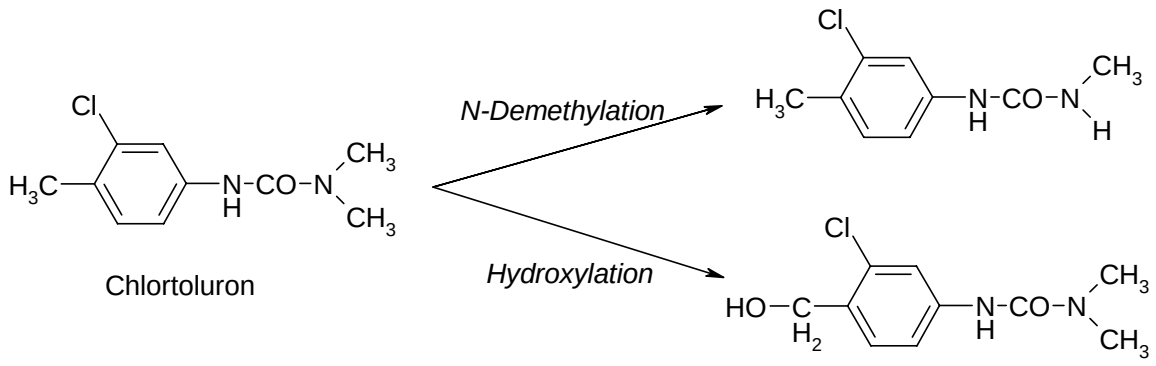


Figure 39. N-Demethylation and ring-methyl hydroxylation of chlortoluron.

After hydroxylation both products undergo conjugation with glucose by the newly formed hydroxyl group.

For some phenylurea herbicides in the Jerusalem artichoke cytochrome P450-mediated N-demethylation is is sufficient to cause partial or complete loss of phytotoxicity (Didierjean et al. 2002; Figure 40).

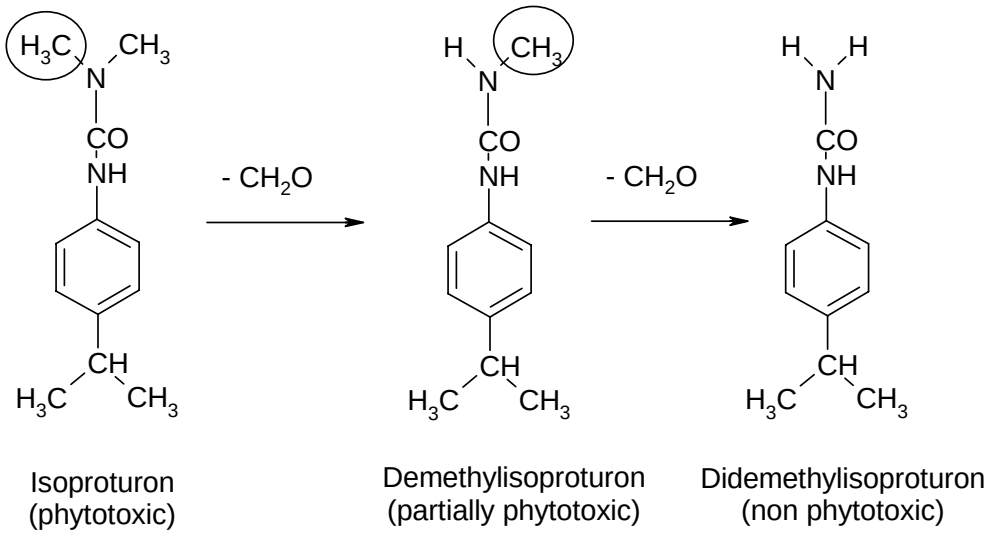


Figure 40. N-demethylation of isoproturon.

Sulfonylurea herbicides (primisulfuron, chlorosulfuron and triasulfuron) in wheat and maize undergo hydroxylation of the aromatic ring (Schuler, 1996; Figure 41).

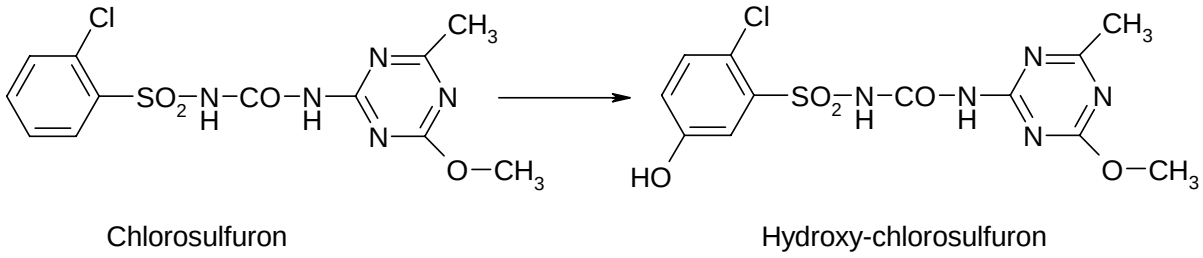


Figure 41. Aromatic hydroxylation of chlorosulfuron.

Analogously, P450-catalyzed transformations are characteristic for other herbicides, that contain an aromatic ring, e.g. dichlofop in wheat (McFadden et al. 1989) and bentazon in maize (McFadden et al. 1990):

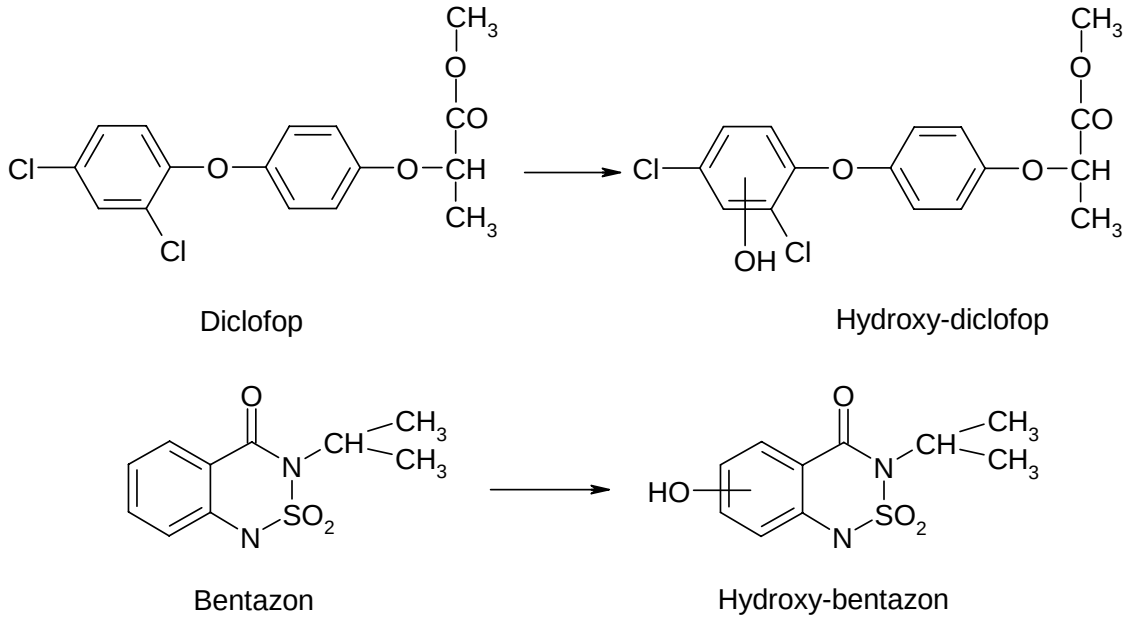


Figure 42. Aromatic ring hydroxylation of dichlofop and bentazon.

Similar to chlortoluron metabolites, after the hydroxylated products of diclofop and bentazon are conjugated to O-glucosides.

The hydroxylation of endogenous substrates and of xenobiotics may be catalyzed by the same cytochrome P450. The corroborations of this suggestion are: oxidation of the endogenous lauric acid and the exogenous diclofop by the cytochrome P450 monooxygenase from wheat (Zimmerlin, Durst, 1992), the endogenous *trans*-cinnamic acid and the exogenous *p*-chloro-N-methylaniline by a recombinant artichoke CYP73A1 (*trans*-cinnamic acid-4-hydroxylase) expressed in yeast (Pierrel et al. 1994). During simulataneous incubation of a microsomal suspension (from etiolated soybean seedlings) with [1-^{14}C] *trans*-cinnamic acid (endogenous substrate) and N,N-dimethylaniline (model xenobiotic) the hydroxylation of the endogenous substrate (according to inconverted cinnamic acid) was inhibited up to 70-80% (Gordeziani et al. 1987). On the other hand,

the demethylation of DMA was inhibited only by 25-30%. Besides N,N-dimethylaniline, the enzymatic transformation of cinnamic acid was also inhibited by other xenobiotics (ethylmorfin, *p*-nitroanisole, aniline and aminopyrine). Kinetics of NADPH-dependent oxidation of cinnamic acid and xenobiotics revealed the competitive character of the inhibition of the cinnamic acid-hydroxylase activity by xenobiotics (Khatisashvili et al. 1997). These results indicated the decrease in cytochrome P450 biosynthesis and its switch to detoxification of xenobiotics. The switch of an enzyme from biosynthesis to detoxification is determined by the polarity of the xenobiotic: the more hydrophobic the xenobiotic, the higher its affinity for P450, the more universal the switch, and the faster the process of oxidation. Thus, the penetration of hydrophobic xenobiotics in the plant cell is a regulatory signal for cytochrome P450 to switch from an "endogenous" to an "exogenous" function regime. In essence, the switch is set into motion by the superior affinity of the xenobiotic for the enzyme compared to its natural substrates.

When plants grow in a medium that contains a xenobiotic, the concentration of cytochrome P450 increases. Nearly all xenobiotics examined have an inductive nature. The inductive abilities of such xenobiotics as phenobarbital, clofibrat, aminopyrine, and 2,4,-D and herbicides propanil, chloracetamide, thiocarbamate, chlortoluron, bentazon, and others (Salaün, 1991) have beennoted. A cytochrome P450 (CYP76B1) was isolated from Jerusalem artichoke, that is more strongly induced by xenobiotics than other cytochrome P450 containing monooxygenases. This CYP76B1 (**CYP** designates **CY**tochrome **P**450, **76** designates the gene family, **B** designates the gene subfamily and **1** designates particular gene) metabolizes with high efficiency a wide range of xenobiotics, including alkoxycoumarins, alkoxyresorufins, and several herbicides of the phenylurea class (Robineau et al. 1998). CYP76B1 catalyzes also the removing of both N-alkyl groups of phenylureas with turnover rates comparable for physiological substrates and produces non-phytotoxic compounds. This P450-increased herbicide metabolism and tolerance can be achieved by ectopic constitutive expression of CYP76B1 in tobacco and Arabidopsis (Didierjean et al. 2002). Transformation with CYP76B1 brought about in tobacco and Arabidopsis a 20-fold increase in tolerance to the herbicide linuron, a 10-fold increase in tolerance to herbicides isoproturon or chlortoluron. Besides increased herbicide tolerance, the expression of CYP76B1 brings no other visible phenotype about in the transgenic plants. CYP76B1 can function as a selectable marker for plants that can be selected for the phytoremediation of contaminated sites.

The inductive effect of each particular xenobiotic depends on its chemical nature and the inductive abilities of the intermediate metabolites. Some of intermediates appear to be reactive and most of them cause the inactivation of cytochrome P450 and its further conversion into cytochrome P420. Good examples of these intermediates are N,N-dimethylaniline and 3,4-benzoapyrene. Growth of soybean and ryegrass in N,N-dimethylaniline-containing solution leads to the intensive induction of an active form of a hemoprotein. N,N-Dimethylaniline oxidation (N-oxidation, N-demethylation, p-hydroxylation) metabolites cannot provide such "active" intermediates as 3,4-benzoapyrene oxidation. Incubation of plants with 3,4-benzoapyrene causes the formation of epoxides, dioles and quinones (Sandermann, 1994). The "aggressiveness" of these substances is expressed by the formation of active oxygen radicals, that cause the

irreversible conversion of cytochrome P450 (Khatisashvili et al., 1997). For instance, enhancement of the peroxidation of fatty acids also leads to the generation of oxygen radicals. According to unpublished data of the authors, during cytochrome P450-mediated 3,4-benzoapyrene oxidation by maize seedling microsomes superoxide anion-radicals are generated.

The expression of genetically ngineered cytochrome P450 would be needed for the low-cost production of several natural products, such as antineoplastic drugs (taxol or indole alkaloids), nutraceuticals (phytoestrogens) and antioxidants in plants (Morant et al. 2003). These compounds may have important functions in plant defense. Engineered cytochrome- P450s could improve plant defense against insects and pathogens. These P450s may be tools to modify herbicide tolerance, and are selectable markers and for bioremediation.

Peroxidases

Peroxidases (EC 1.11.1.7) are ubiquitous enzymes found in virtually all green plants, the majority of fungi and aerobic bacteria. The isozymic heterogeneity of peroxidases appears to result from *de novo* synthesis, as well as an array of physiological and ecological determinants including hormones, light, gravity, and infection (Siegel 1993). Peroxidases have phylogenetically-correlated similarities based on the chemical nature and redox potentials of the substrates which they can oxidize. Peroxidases often increase in response to stress, and one of principal roles of peroxidases appears to be to protect cells from oxidative reactions imposed on all photosynthetic plants. The great catalytic versatility of the peroxidase is its predominant characteristic, and, therefore, no single major role exists for this multifaceted enzyme.

The peroxidase is defined by the reaction:

$$RH_2 + H_2O_2 \rightarrow 2H_2O + R$$

It is composed of a single peptide chain, contains one heme (protoporphyrin IX), and the plant enzyme (as distinct from the animal peroxidases) is about 25% carbohydrate which protect it from proteolytic degradation and stabilizes the protein conformation (Hu, Van Huystee, 1989).

The peroxidases are known to catalyze a number of free radical reactions (Stahl, Aust, 1995). The "resting" enzyme (ferric-heme protein) is initially oxidized by two electrons in a reaction with hydrogen peroxide. An early step in the catalytic cycle following the binding of hydrogen peroxide to the heme in the Fe(III) state, is the heterolytic cleavage of the O–O bond in hydrogen peroxide. Two key catalytic residues in the distal heme pocket, an arginine (Arg38) and a histidine (His42), are involved in peroxide activation and compound I formation by catalyzing proton transfer from the α- to the β-oxygen atom of heme-bound H_2O_2 and polarizing the O–O bond. His42 acts initially as a proton acceptor (base catalyst) and then as a donor (acid cataliyst) at neutral pH. Arg38 is influential in lowering pK_a of His42 and additionally in aligning H_2O_2 in the

active site, but it does not play a direct role in proton transfer (Rodrígez-López et al. 2001). The resulting intermediate, compound I, is an oxyferryl (Fe^{IV}) heme protein with a cation radical stabilized in the heme porphyrin ring. Compound I is then converted back to the resting enzyme via two successive single-electron transfers from reducing substrate molecules. The first reduction, on the porphyrin radical cation, yields a second enzyme intermediate, compound II, which retains the heme in the ferryl state (Ferryl enzyme. Compound II can be reduced again to regenerate ferric enzyme or react with hydrogen peroxide to form a catalytically inactive species, compound III (ferric-superoxide protein). Alternatively, a compound that is oxidized directly by the enzyme can oxidize other chemicals.

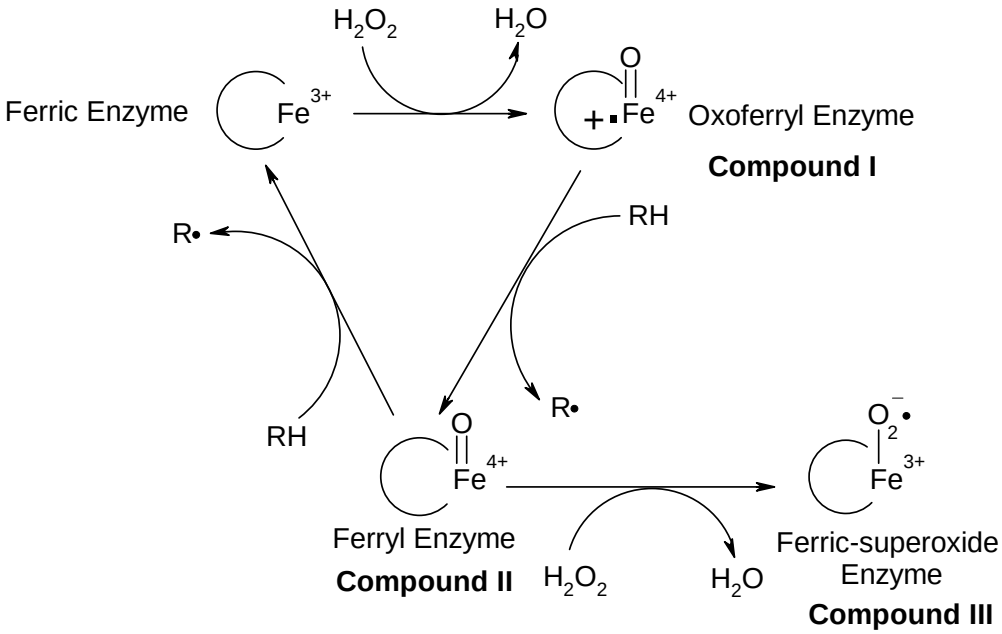


Figure 43. The action mechanism of peroxidase

Analogously also peroxidases of microorganisms participate in the oxidative reactions: lignin peroxidase (LiP) and manganese dependent peroxidase (MnP). In addition to catalyzing the oxidation of different compounds, LiP is also able to catalyze reductive reactions in the presence of electron donors such as EDTA and oxalate (Stahl, Aust, 1995). …The electron donors, such as veratryl alcohol (a free radical mediator) is oxidized by a LiP and generate cation radicals. As a result, the anion radical can catalyze the reduction of electron acceptors (cytochrome c, nitroblue tetrazolium, O_2). Similar reactions have been observed in the case of MnP in the presence of quinones. These reductive mechanisms may be involved in the metabolism of TNT in *Phanerochaete chrysosporium*, but it is shown that they are not participating in the initial reducing steps of this explosive (Stahl, Aust, 1995).

One hypothesis states that organic contaminants in plants are oxidized by peroxidases (Stiborova, Anzenbacher, 1991). This hypothesis is based on a such arguments, as an ubiquitous occurrence of this enzyme in plants (its' isozymes in green

plants occur in the cell walls, plasmalemma, tonoplasts, intracellular membrane systems of endoplasmic reticulum, plastids and cytoplasm), its' high affinity to organic xenobiotics of different chemical structure, low substrate specificity, etc. This feature ensures the active participation of peroxidase in a wide variety of detoxification processes. The results of many investigations also indicate the participation of plant peroxidases hydroxylation reactions of xenobiotics. For example, peroxidases from different plants are capable to oxidize N, N-dimethylaniline (Shinohara et al. 1984), 3, 4-benzoapyrene, 4-nitro-o-phenylendiamine (Wilson et al. 1994), 4-chloroaniline (Laurent, 1994), phenol, aminoflourene, acetaminophen, diethylstilbestrol, butylated hydroxytoluene, hydroxyanisoles (Sandermann, 1994). According to unpublished data of the authors, horseradish peroxidase can oxidize tritium-labeled [C^3H_3]TNT.

Phenoloxidases

Phenoloxidase (EC 1.14.18.1) is a copper-containing enzyme thatis widely distributed throughout microorganisms, plants, insects and animals (Mayer, 1987; Sugumaran et al. 1999). It is of central importance in such processes as vertebrate pigmentation and the browning of fruits and vegetables. The enzyme exists in multiple forms in active and latent conditions, and catalyzes both the monooxygenase and oxygenase reactions: the *o*-hydroxylation of monophenols (monophenolase reaction) and the oxidation of *o*-diphenols to *o*-quinons (diphenolase reaction) (Sánches-Ferrer et al. 1994).

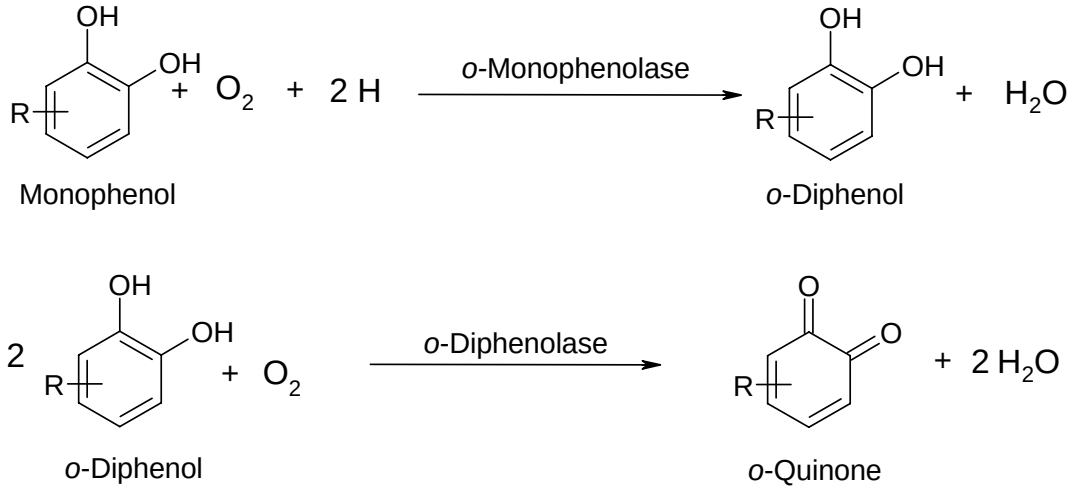


Figure 44. *o*-Monophenolase and *o*-diphenolase activities of phenoloxidases

The catalytic cycles for the monophenolase and diphenolase activities are coupled not only to each other, but also to non-enzymatic reactions involving *o*-quinone products (Rodrígez-López et al. 1992).

The tentative mechanism of the phenoloxidase action is based on results obtained for mushroom (*Agaricus bisporus)* tyrosinase, a typical representative of phenoloxidases.

(Rodrígez-López et al. 2000). The enzyme has three: met-, deoxy- and oxy-forms depending on the state of the two copper ions of the binuclear site, where they are surrounded with six nitrogen atoms of the histidine residues. The met- and oxy- form copper ions are bivalent, and the deoxy- form copper ion is univalent. Besides, substrates (e.g. *o*-diphenol) bind to the met- and oxy- forms, but not only to the deoxy- form. Oxygen can only bind to the free deoxy- form, which does not bind *o*-diphenol.

In the diphenolase activity, the *o*-diphenol binds to the axial position of one (let left) of the copper (II) ions of the met site (E_{met} in the scheme). Coordination of the *o*-diphenol is accompanied by the transfer of a proton to a protein residue represented in scheme by B. Bidentale coordination of the *o*-diphenol is accompanied by a second proton transfer, probably by displacement of an axial histidine coordinated???? to the second (right) copper (II) ion. Electron transfer from the *o*-diphenol substrates results in the formation of the *o*-quinone and the deoxy- form (E_{deoxy}) of the binuclear copper site, where metal ions are in the monovalent state.

In the neutral area which provided by the protonated acid-base catalyst (BH) could favor the rapid binding of oxygen molecule to deoxy- form of phenoloxidase. In the oxy- form the atoms of oxygen are proposed to bind in the peroxide fashion. Further binding of the *o*-diphenol to one copper (II) ion of the oxy- form takes place. After binding, the phenoloxidase oxidizes the substrate to *o*-quinone. That stage is the rate-limiting step of the complete catalytic cycle. It is accompanied by the transfer of a proton to the bound peroxide. Bidentale coordination of the *o*-diphenol substrate is accompanied by a second proton transfer. The transfer of an electron from the *o*-diphenol to the peroxide induces cleavage of the O–O bond to form a water molecule and the *o*-quinone while regenerating the initial met- form of phenoloxidase. In this last step the protein residue B act as an acid, providing a proton for the release of water (Rodrígez-López et al. 2000).

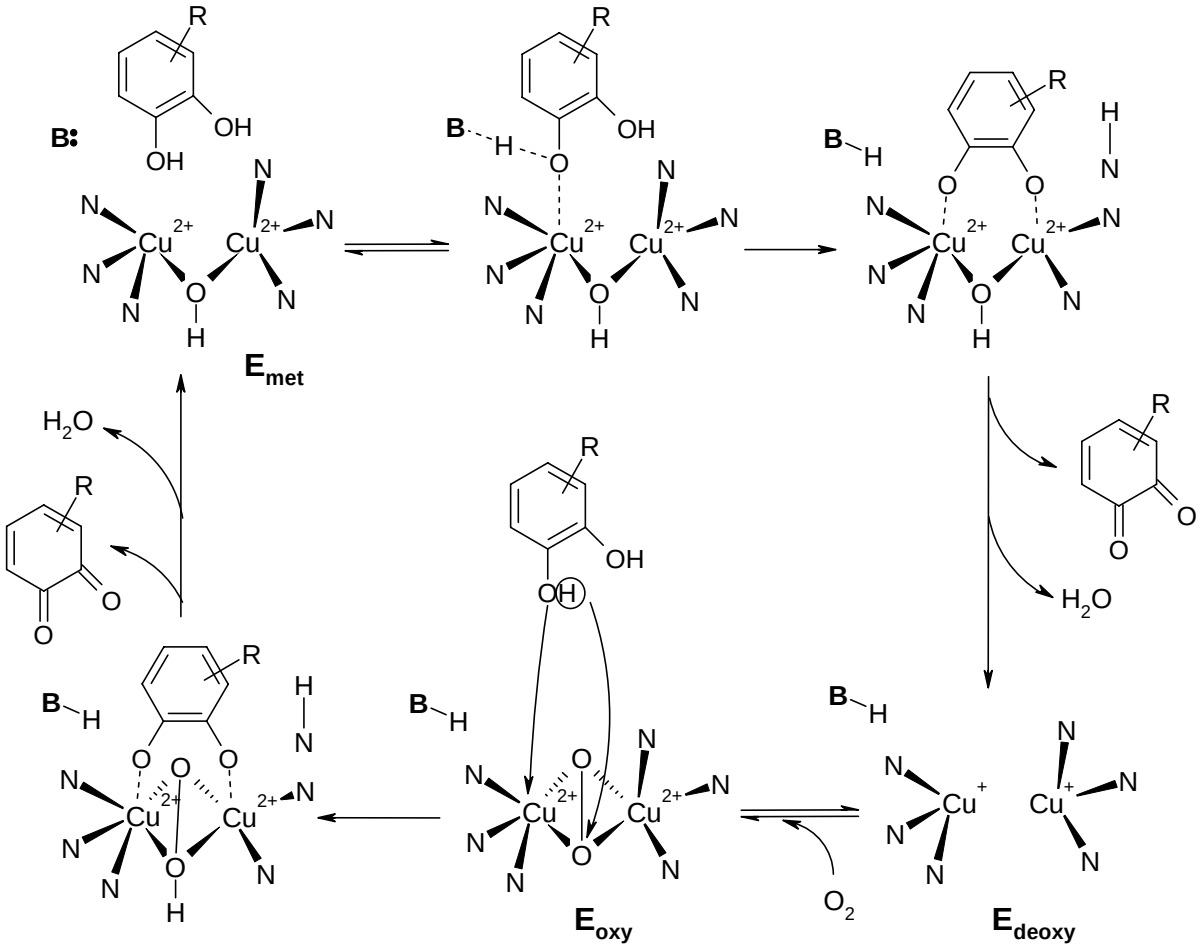


Figure 45. The action mechanism of phenoloxidase.

In addition to its main function, catalyzing the oxidation of phenolic compounds, phenoloxidase also actively participates in the oxidation of xenobiotics. In this process, both enzymes are active, depending on the structure of the substrate. Phenoloxidase from spinach (*Spinacia oleracea)* oxidizes aromatic xenobiotics (benzene, toluene), and is active in their hydroxylation and further oxidation to quinone (Ugrekhelidze et al. 1997). If the xenobiotic subjected to oxidation is not a substrate of the phenoloxidase, the enzyme oxidizes endogenous phenols by forming qiunones and semi-quinones, compounds with a high redox potential. These compounds activate molecular oxygen and forms oxygen radicals, such as superoxide anion radical ($O^{2-\cdot}$) and hydroxyl radical ($^{\cdot}OH$) (Guillén et al., 1997; 2000), that have the ability to oxidize organic xenobiotics. The formation of these radicals enables the phenoloxidase to participate in detoxification processes by the co-oxygenation mechanism, represented below:

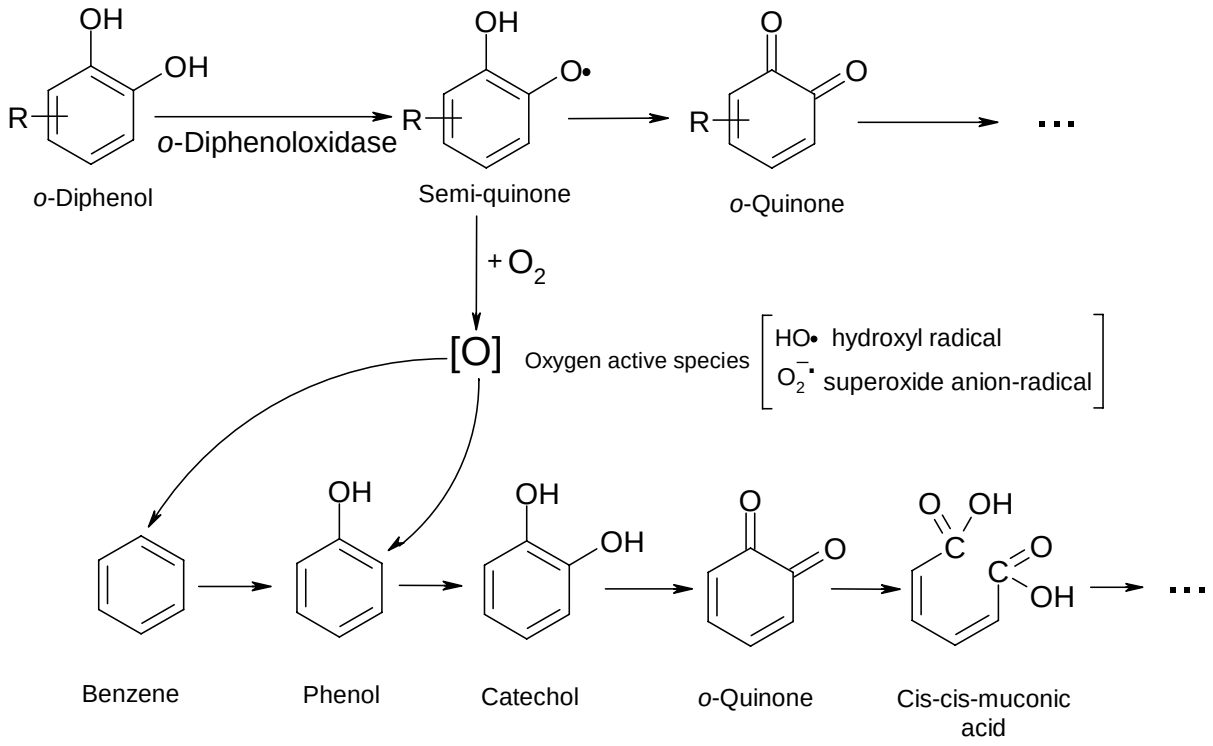


Figure 46. Enzymatic oxidation pheoloxidase (upper) and non-enzymatic co-oxidation of benzene (lower).

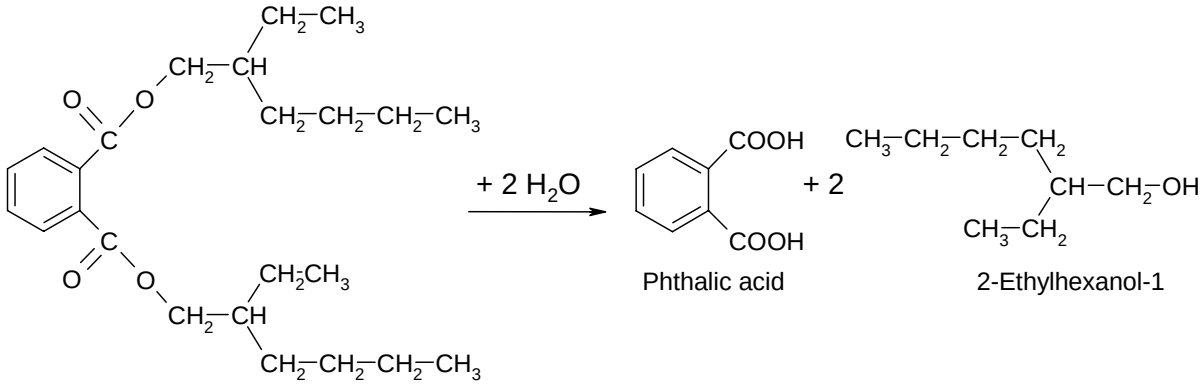
Analogousto the oxidation of nitrobenzene to *m*-nitrophenol, and the methyl group of TNT was oxidized by an enzyme prepared from tea leaves (unpublished data of the authors).

Data about the participation of plant phenoloxidases in the oxidation of xenobiotics is rare (Ugrekhelidze et al. 1997). Laccases of fungi are better explored. Laccases biodegrade many aliphatic and aromatic hydrocarbons (Colombo et al., 1996), and participate in the enzymatic oxidation of alkenes (Niku-Paavola, Viikari, 2000). Crude preparations of laccase isolated from the white rot fungus *Trametes versicolor* oxidize 3,4-benzoapyrene, anthracene, chryzene, phenanthrene, acenaphthene and other PAHs (Collins et al. 1996; Johannes, Majcherczyk, 2000). The intensity of the oxidation of these toxicants increases in the presence of such mediators as: phenol, aniline, 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol. The rate of oxidation increases proportionally to the redox potential of the mediators until E_h<0.9V E_h; The rate decreases at a redox potential of E_h>0.9V. The natural substrates, methionine and cysteine, reduced glutathione, and others also stimulate the oxidation of xenobiotics. These data indicate that in the cases of laccase and o-diphenoloxidase, the oxidation of hydrocarbons is carried out by a co-oxidation mechanism.

Esterases

Several lipophilic organic contaminants containing ester bonds acquire functional groups not only via oxidation, but also via hydrolysis. Among them are such compoundsas phthalate esters (chemical plasticizers), 2,4-D, diclofop methyl, bromoxynil octanoate, binapacryl, aryloxyphenoxypropionate, pyrethrine (pesticides), etc. The functionalization of organic xenobiotics via hydrolysis is catalyzed by serine hydrolases such as carboxylesterases (EC 3.1.1.1) (Krell, Sandermann, 1985; Sandermann, 1994; Cummins et al. 2001; Cummins, Edwards, 2004). These enzymes from microsomes have a wide specificity and in addition to their basic reaction (hydrolysis of carboxyl ester with formation of an alcohol and a carboxyl acid) also catalyze the reactions of EC 3.1.1.2 (arylesterase), EC 3.1.1.5 (lysophospholipase), EC 3.1.1.6 (acetylesterase), EC 3.1.1.23 (acylglycerol lipase), EC 3.1.1.28 (acylcarnitine hydrolase), EC 3.1.2.2 (palmitoyl-CoA hydrolase), EC 3.5.1.4 (amidase) and EC 3.5.1.13 (aryl-acylamidase), etc. This specificity allows the esterases to actively participate in the phase of functionalization of lipophilic xenobiotics.

One of 12 non-specific esterases in wheat showed a preference for a substrate chain-length of 6-8 carbon atoms, and this form of esterase was active with the plasticizer chemical bis(2-ethylhexyl)phthalate (Krell, Sandermann, 1984). The potential cleavage pathway of this molecule is presented in Figure 47.:



bis(2-Ethylhexyl)phthalate

Figure 47. Hydrolysix of bis(2-Ethylhexyl) phthalale by esterase.

Esterases also effectively hydrolyze model xenobiotics such as *p*-nitrophenyl acetate and α-naphthyl acetate. Comparison of various plant esterase activities showed that the activity itowards model xenobiotics was the highest in wheat, while those in weeds (wild oat (*Avena fatua*), black-grass (*Alopecurus myosuvoides*)) were more active in hydrolyzing the pesticide esters (diclofop methyl, bromoxynil octanoate, binapacryl) (Cummins et al. 2001). This distinction is caused by different forms of esterases in plants. All weeds contain the more basic esterases (pI>5.0) with a high affinity towards

pesticides, while the acidic esterase (pI 4.6) from wheat has the greatest activity toward α-naphthyl acetate but was insensitive to pesticides.

The hydrolase superfamily in plants is important for the endogenous metabolism and herbicide bioactivation in crops and weeds. A member of the family of serine hydrolases (designated by GDSH), carboxyesterase, which activate aryloxyphenoxypropionate graminicides to their bioactive herbicidal acids by hydrolysing the respective ester precursors have been identified in black-grass (*Alopecurus myosuroides*), a problem weed of cereal crops in Northern Europe (Cummins, Edwards, 2004). This enzyme (designated by *Am*GDSH1) was cloned and expressed in the yeast *Pichia pastoris* as a secreted enzyme. Expression was associated with activity towards aryloxyphenoxypropionate esters. *Am*GDSH1 was predicted to be glycosylated and exported to the apoplast *in planta*.

Nitroreductases

The enzymes catalyzing the reduction of the nitro groups in explosives such as 2,4,6-trinitrotoluene (TNT) are EC 1.6.6 non-specific NAD(P)H dependent nitroreductases (Esteve-Núñez et al. 2001). These enzymes are found in animals, plants and microorganisms.

The apoenzyme nitroreductase from *Enterobacter cloacae* contains two monomers and binds two flavin mononucleotide prosthetic groups at the dimer interface (Haynes et al 2002). The enzyme derives reducing equivalents from NADH and NADPH by means of two flavin mononucleotide cofactors (FMN). In the oxidized enzyme, the flavin ring system adopts a strongly bent (16°) conformation, and the bend increases (25°) in the reduced form of the enzyme, roughly the conformation predicted for reduced flavin free in solution. Free oxidized flavin has a planar configuration, the induced bend in the oxidized enzyme may favour reduction, and it may also account for the characteristic inability of the enzyme to stabilize the one electron-reduced semiquinone flavin, which is planar.

The transformation of TNT in many respects is predetermined by its original chemical structure. The polarization of the N–O bond due to more electro-negativity of oxygen then nitrogen induces partially the positive charge of the nitrogen. Hence, this charge combined with the high electronegativity of nitrogen makes the nitro group easily reducible. On the other hand, the delocalized π electrons from the aromatic ring of TNT are removed by the electronegative nitro groups that make this ring electrophilic (Preuss, Rieger, 1995).

To perform this reaction, nitroreductase uses reduced pyridine nucleotides (both NADH and NADPH) as electron source (Zenno et al. 1998). There are two types of nitroreductases (Esteve-Núñez et al. 2001). Type I, present in animals, plants and a number of microorganisms (e.g. strains of *Bacillus*, *Staphylococcus*, *Actinomycetes*, *Pseudomonas*, etc.), reduces the nitro group by two-electron transfers. This pathway is oxygen independent and no radicals are formed (Bryant et al. 1981). Therefore the

nitroreductases of type I are active in both anaerobic and aerobic conditions. Type II are oxygen sensitive nitroreductases and these reduce the nitro groups through single-electron transfers, forming a nitro-anion radical. In aerobic conditions an oxygen molecule reacts with a nitro-anion radical and forms a superoxide anion radical that makes the process of TNT transformation reversible. Such nitroreductases are found in rat liver microsomes, and in strains of *Escherichia coli* (Peterson et al. 1979) and *Clostridium* (Angermaier, Simon, 1983).

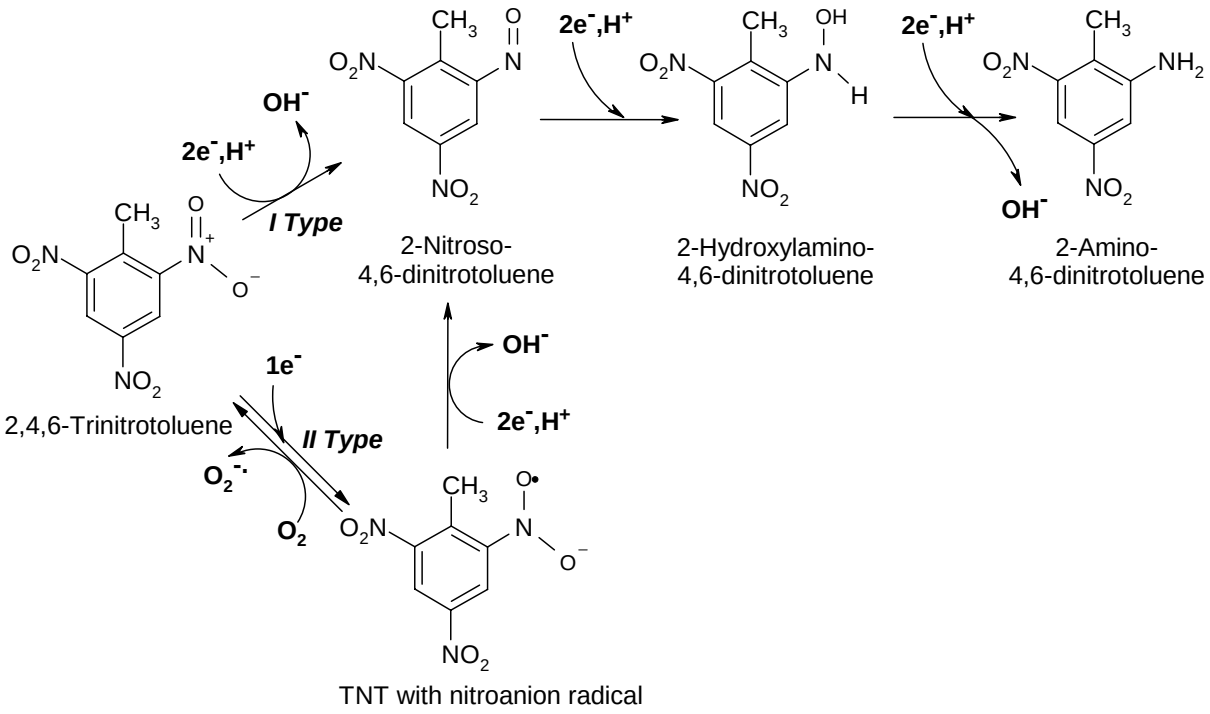


Figure 48. TNT reduction by two types of nitroreductases.

The reduction of the first nitro group in TNT is generally much more rapid than of the rest. The conversion of the nitro- to amino- groups decreases the electron deficiency of the nitroaromatic ring, and consequently a lower redox potential is required to reduce the rest of the nitro- groups of the molecule.

Nitroreducatases catalyze further transformations of the other nitro groups of TNT to amino groups. It is also possible that removal of the nitro- group from the *o*-position and following reduction of removing nitrite ion by nitrite reductase. As indicated above, the electron deficiency in the aromatic nucleus of TNT induces a nucleophilic attack on this molecule. The hydride anion from the reduced pyridine nucleotides attacks the aromatic ring, and as a result a non-aromatic structure such as a Meisenheimer σ complex can be formed (Fant et al. 2001). Further, a nitrite anion is released from the Meisenheimer complex with the formation of dinitrotoluenes. Oxygen is not required for the formation of this compound, and thus this process is an alternative for the metabolism of nitroaromatic compounds when oxidative removal of the nitro groups is not possible.

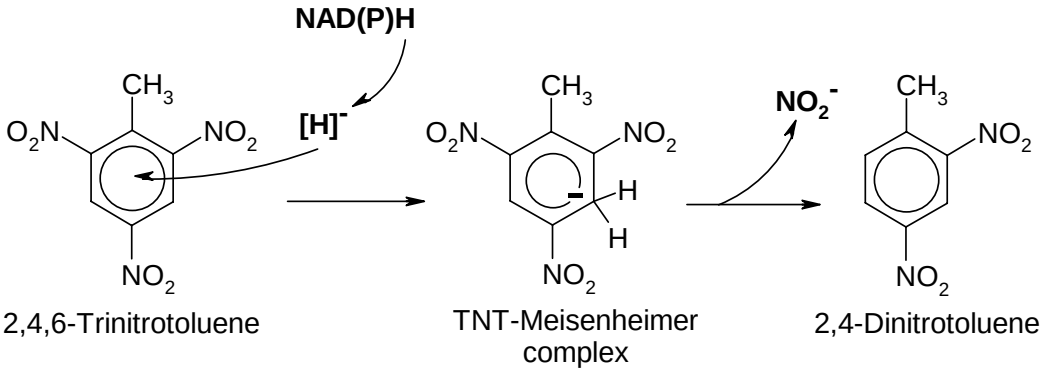


Figure 49. The remove of nitro group from TNT.

The nitro, nitroso and hydroxylamino groups are responsible for the toxicity and mutagenic activity of TNT and its derivatives. It has also been shown that complete reduction of the nitro groups to amino groups decreases the mutagenic effect of this compound (Cash, 1998).

The presence of a highly active nitroreductase is required for plants that are used to phytoremediate explosives-contaminated soils and ground-waters. The correlation between the plant nitroreductase activity and ability to absorb TNT from aqueous solutions has beendemonstrated, and the higher the nitroreductase activity, the faster the assimilation of TNT by the plant (Khatisashvili et al. 2003). These results support the hypothesis that plant nitroreductase activity may serve as a simple preliminary biochemical test to select plants with a potential for the phytoremediation of areas contaminated with TNT. Some plants actively absorband transform TNT: yellow nutsedge (Palazzo, Legget, 1986), bush bean (Harvey et al. 1990), switchgrass (Peterson et al. 1998), parrot feather (*Myriophyllym aquaticum*), stonewort (*Nitella*), algae, ferns, monocotyledonous and dicotyledonous plants, aquatic and wetland species (Best et al. 1997), hybrid poplar (Thompson et al. 1998), soybean (Khatisashvili et al. 2003). In the transformation of TNT by plants the formation of monoamino derivatives 2-amino-4,6-dinitrotoluene and 4-amino-2,6-ddinitrotoluene takes place. Large part, sometimes about 60%, of the metabolites seems to be involved in conjugation with insoluble biopolymers (Sens et al. 1999; Bhadra et al. 1999; Unpublished data of the authors). These conjugates are compartmentalized into the vacuoles and cell wall.

The use of a plant-bacterial consortium (e.g. *Pseudomonas* strain together with meadow bromegrass (*Bromus erectus*)) for the phytoremediaton of contaminated soil has also been published (Siciliano et al. 2000). The bacteria have an active nitroreductase able to transform TNT into its monoaminodinitrotoluene and diaminonitrotoluene metabolites that promotes the removal of explosives from soil by plants.

Transgenic plants with a gene of a microbial nitroreductase have also been created for the phytoremediation of TNT (French et al. 1999). A transgenic tobacco plant with an

expressed gene of a bacterial nitroreductase acquired the ability to absorb and eliminate TNT from the soil of military proving grounds (Hannink et al., 2001).

Transferases

Enzymes classified as transferases (EC 2) are responsible to catalyze reactions after functionalization, in the phase II of transformation, that are accomplished by conjugation of the parent toxic compounds and/or their degradation intermediates with endogenous plant cell constituents. The participation of separate enzymes depends on chemical nature of the intermediates and on the existence of the neededcell consitutents. According to the literature data the conjugation is carried out via Glutathion-S-transferases (EC 2.8.1.5), O-glucosyl-transferases (EC 2.4.1.7), N-glucosyltransferase (EC 2.4.1.71), N-malonyltrasferases (2.3.1.114), putrescine N-methyl-transferase (2.1.1.53), etc (Sandermann, 1994). These enzymes are needed for normal plant cell metabolism and are involved in the xenobiotics transformation in cases where toxic compounds are involved. All cell constituents that bind with the toxicants in theconjugation processes have a hydrophylic character and ,thus, thelipophilicity of the toxicants decreases significantly. Therefore, the conjugates are somewhat more soluble in the cytoplasm and undergo compartmentalization.

The group of enzymes of wide specificity glutathion-S-transferases (other names: Glutathione S-alkyltransferase, Glutathione S-aryltransferase, S-(hydroxyalkyl)-glutathione lyase, Glutathione S-aralkyltransferase) connect the electrophilic toxicants and their metabolites with reduced tripeptide glutathione (γ-Glu–Cys–Gly). The glutathione transferases are encoded by a large and diverse gene family in plants, which can be divided on the basis of their sequence identity into the π (pi), τ (tau), θ (theta), ζ (dzeta) and λ (lambda) classes (Dixon et al. 2002). Glutathion-S-transferases provide a reaction between the functional group of the toxicant intermediate and the SH-group of the glutathione cysteine residue. As a result, the toxicant binds via covalent bonds with the sulfur atom(Fig. 50)

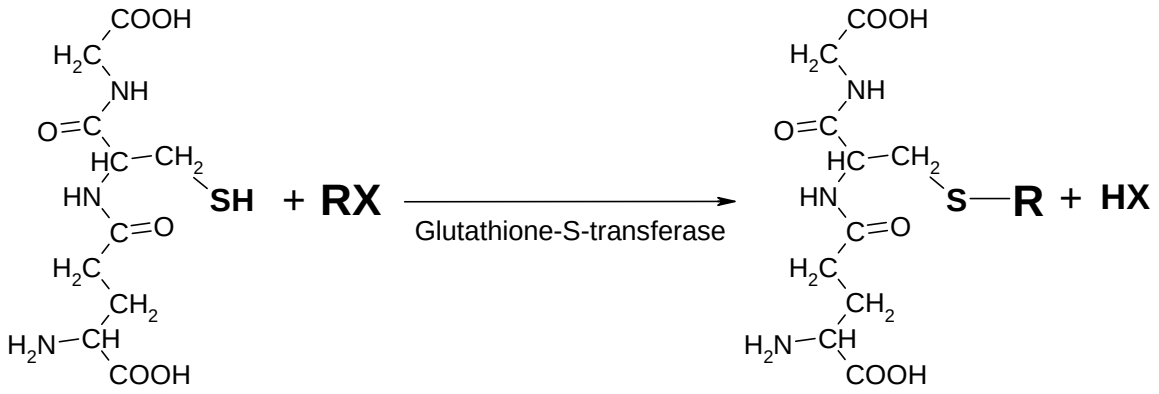


Figure 50. Conjugation of toxicant with reduced glutathione.

R may be an aliphatic, aromatic or heterocyclic group. *X* may be a sulfate or nitrite group, etc. The glutathion-S-transferases also catalyze the addition of aliphatic epoxides and arene oxides to glutathione; the reduction of polyol nitrate by glutathione to polyol and nitrite; certain isomerization reactions and disulphide interchange.

The glutathion-S-transferases form an important part of the cellular detoxification system and are found not only in plants but also in most organisms. They participate in conjugation of a wide spectrum of toxic compounds such as herbicides: FOE 5043, triflusulfuron, chlorimuron-ethyl, acetochlor, metolachlor, alachlor, atrazin (Bieseler et al. 1997), safeners (DeRidder et al. 2002), fluorodifen (Dixon et al. 2003), etc.

Glucosyltransferases catalyze the reaction between glucose and hydroxyl groups (O-glucosyltransferases) or amino groups (N-glucosyltransferases) of xenobiotics (Loutre et al. 2003). Both enzymes are inducable by the action of some herbicides (e.g. safeners) and organic pollutants (e.g. 3,4-dichloroaniline, 4-nitrophenol and 2,4,5-trichlorophenol) (Brazier et al. 2002).

In different plants toxicants can undergo conjugation by different transferases. For example, the herbicide 3,4-dichloroaniline can be metabolized via N-malonyltransferase in soybean root cultures, but via *N*-glucosyltransferase in *Arabidopsis thaliana* root cultures (Lao et al. 2003):

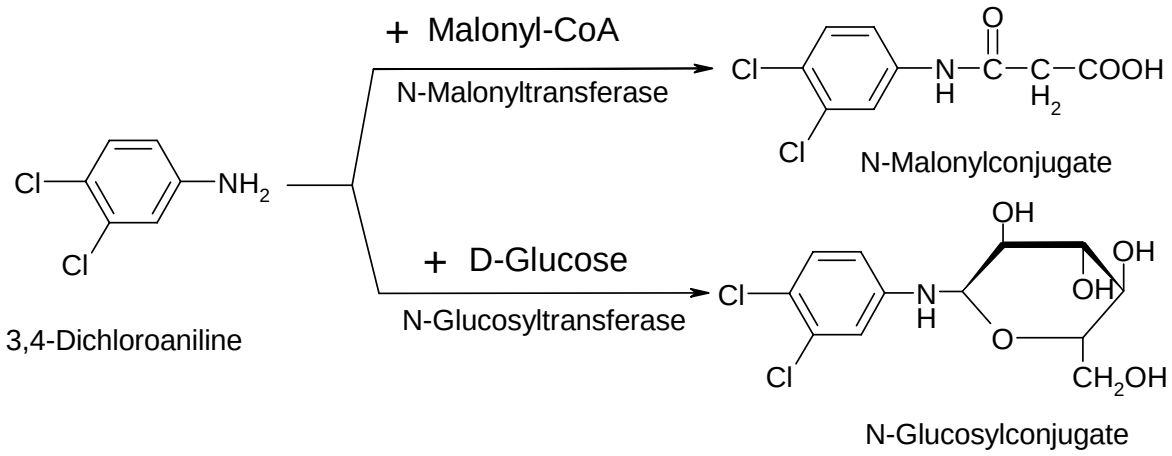


Figure 51. Two pathways of 3,4-dichloroaniline conjugation.

2,2-Bis-(4-chlorophenyl)-acetic acid (DDA), the first intermediate of the insecticide DDT metabolism in soybean was conjugated by the formation of O-glucoside (Sandermann, 1994). It was calculated that the conjugation capacity of soybean O-glucosyltransferase is 855 µg DDA per h per g fresh weight of cells (Wetzel, Sandermann, 1994).

10 - ACTION ON THE CELL STRUCTURE

Electron microscopes, fixation of tissues and ultra thin sections allow the investigation of cells at the subcellular level and determination of negative effects of toxic contaminants in the environment.

Multiple investigations show that complex morphological changes, connected with the destruction of the ultrastructural architecture of the cell are evoked by organic contaminants(pesticides, hydrocarbons, phenols, aromatic amines etc) in plant cells (Durmishidze 1988; Kumar, Subrash, 1990; Allnuf et al., 1991; Buadze, Kvesitadze, 1997; Buadze et al. 1998; Zaalishvili et al. 2000; Korte et al 2000). The destruction of cell organelles by the action of toxicants promotes various pathological processes to proceed, changes in theintensity of vitally important processes and becomes the initial cause of cell death.

The photosynthetic apparatus is most sensitive to the action of the contaminants and the intermediates of their primary transformations (Sharma et al., 1989; Furikawa, 1991). Toxicants may inhibit enzymes involved in the the tricarboxylic acid cycle and in the process of oxidative phosphorylation, and block the biosynthesis of ATP and other energetically important nucleotides: ADP, AMP, GTP, etc. (Bataynen et al., 1986). However, the reaction of the plant cell to the penetration of the toxic compound depends on its' chemical nature, concentration and duration of toxicity.

Changes inCell Ultrastructure Caused by the Action of Organic Contaminants

Estimates of the state of the cell ultrastructural organization during the action of the toxicant allows the determination of the toxic dose for each plant and evaluation of their detoxification potential (Zaalishvili et al., 2000; Korte et al., 2000). Sequence and characteristics of the destruction of the cell organelles depend on the structure, concentration and duration of the action of the toxic compound, the sensitivity of the cell, the rate of adaptation and many other factors (Buadze et al., 1998).

Studies on the penetration ^{14}C–labled toxic compounds into the cell show, that labeled toxicants at the early stages of exposure (5–10 min) are detected in the cellular membrane, in small amounts in nuclei and nucleolus, and seldom – in the cytoplasm and mitochondria. After longer exposure, the toxicant amount significantly increases in the nucleus, on the membranes of organelles and of the tonoplast, and further in vacuoles (Zaalishvili et al., 2000), i.e. toxic compounds with different structures are characterized by their distribution in all subcellular organelles, but finally there is tendency to accumulate in the vacuole.

Ten-min exposure of maize seedlings to 1 mM solution [1-^{14}C] of **phenoxyacetic acids** caused the insertion of the herbicide label in nucleus, nucleolus and vacuoles of cells of the root apex. At that time the radioactive label occupied about 3% of the total cell area. In sunflower within the same period of time THE label of [1-^{14}C]

phenoxyacetic acid occupied over 16% of the apex cells, although unlike in maize, the toxicant was concentrated in the intracellular space and less in the nucleus (Fig.52). Analogous to sunflower, phenoxyacetic acid penetrated the cellular membrane of pea root apex cells in large amounts, but in this case the toxicant was localized in the cytoplasm, nucleus and nucleolus.

Maize cells are less permeable for labeled [1-^{14}C] **2,4-D** than to phenoxyacetic acid. 2,4-D penetrates more easily and deeply in pea and sunflower cells and is then localized in different subcellular organelles. Thirty-min incubation of different plants with this toxicant showed that in maize cells the herbicide ended up in the nucleus or vacuoles, in small amounts in the cytoplasm and membranes, occupying less than 5% of the total cell. In sunflower and pea the radioactive label of the toxicant occupied more than 30% of the intracellular space, by ending up in the mitochondria, plastids, nucleus and nucleolus (Buadze et al. 1985, 1986). The lowest permeability for toxicants was found in maize (7%), the average in pea 22%, and the highest was 50%.

Incubation of the aromatic amine [1-6-^{14}C] **benzidine** (concentration 0.22 x10 $^{-4}$mM) with roots of a 3-day old maize seedlings led after 10 min of exposure to penetration of the toxicant in the cells. Within this period the benzidine passed the cell wall, plasmalemma and translocated into the nucleus. After 30 min, the toxicant penetrated into the nucleolus, and after 60 min the plant cell began to struggle with the toxicant and most of benzidene was accumulated in the vacuole.

The penetration of [1-^{14}C] **benzoic acid** proceeded far more slowly in plants. This toxicant at a concentration of 1mM reached the nucleus in 1h. After 24 h, the radioactive label occupied almost the whole cell, i.e., cytoplasm, plastids, mitochondria, endoplasmic reticulum and tonoplasts of vacuoles, but in the nuclei its content was significantly reduced. After 72 h, the radioactive label accumulated largely in the vacuole, while insignificant amounts remained in the other organelles and cytoplasm.

The general picture of the actions of organic contaminants on plant cells is the following:

- Initially, a change in the configuration of the nucleus occurs. An inhibition of DNA synthesis (Zaalishvili et al., 2000) takes place. The barrier function of the plasmalemma and its ability to accumulate calcium are damaged. The Ca^{2+} concentration in the cytoplasm is enhanced (Korte et al., 2000) and the Ca^{2+} -ATP-activity is inhibited. In cells, that undergo the action of a toxicant mitochondria with swollen crists and packed matrix (Fig. 53)are found, the plastids are electron-dense and are enlarged .

- Prolonged action of a toxicant leads to a widening of cisterns of endoplasmic reticulum and Golgi apparatus, and vacuolization of the cytoplasm The size of cytoplasm is decreased and the periplasmatic space is enlarged. In some cortex cells of the root apices, the number of ribosomes in the hyaloplasm is increased, and polysome formation is observed. Lysis of the mitochondria, run down of the

ribosomes from the membranes of endoplasmic reticulum take place. Multiple contacts between endoplasmic reticulum and plasmalemma, plasmodesma vacuoles, nucleus, membranes and mitochondria are detectable. It has been observed the enhancement of size of nucleus and chromatin coagulation indicating a distrubance of the process of DNA synthesis. Nuclei acquire deviating shapes becauee of the development of many protuberances of the nucleus membrane (Fig. 54). In leaf cells the shape and composition of the chloroplast acquiring indefinite configuration are observed, the external membrane is not visible, the orientation of the system is disturbed, the matrix is lightened with large osmiophilic inclusions. In the cytoplasm of the differentiated cells of root caps, that secrete the mucus accumulation of hypertrophied secretary vesicles is visible, most of which remain at the place of their formation or stay connected with the cytoplasm organelles (e.g. mitochondria) instead of translocating to the periphery and fussion with plasmalemma. Some of these hypertrophied vesicles are fused forming a large depot of mucus. Inhibition of the process of translocation of maturing secreting vesicles towards the cell periphery is often correlated not only with the swelling of vesicles, but also with the disappearance of the normal dictiosomes.

- Longer exposure to toxicants causes strong destruction of the cell and plant death.

Based on numerous experiments the effects of different toxicants on the cells ultrastructural organization can be divided in three levels depending on its concentration:

1. At a **metabolic concentration** in the cell, no deviations from the norm are visible. This dose corresponds to the concentration of the organic toxicant that occurs most frequently under natural conditions.

2. **A concentration inhibiting cell processes** leads to visible changes in the cell ultrastructure, damage of the synthesis of nucleic acids and calcium homeostasis.

3. **A lethal concentration** leads to the total cell destruction and plant death.

The Effects of Low-molecular Alkanes and Alkenes

The actions of different chemical classes of toxicants on the ultrastructural organization of the plant cell have been investigated. In all experiments it was demonstrated that toxicants negatively affected the structural organization and inhibited the metabolic processes of the plant cell. The effect of low concentrations of saturated and unsaturated hydrocarbons on the ultrastructure of different plant leaves has been studied by the authors (unpublished). Maize seedlings were incubated for 48 h in hermetic growth chambers with an atmosphere containing 25% (by volume) hydrocarbon.

In case of **methane**, the following morphological changes have been observed in the epidermal cells of maize seedling leaves: the chloroplasts were distributed on the periphery of the cell and starch had accumulated significantly. Vacuolar bubbles were visible on the external membranes of the chloroplasts(Fig. 55). Elongated lamellar membranes were stretched along the whole chloroplast over the longitudinal long axis. Large quantities of lipid insertions as well as mitochondria with an electron dense matrix and widened cristae were concentrated around the chloroplasts. Completely destructed chloroplasts without double membranes were noticeable in some cells. Separate grains of these chloroplasts were split and located in the lightened matrix of cytoplasm.

The chloroplasts of the cells in the middle part of the maize leaf were crescent shaped. In most cases the chloroplasts were grouped along the cell periphery. In some cells destroyed grains and chloroplasts with lysed internal membranes were visible. The mitochondria had lightened or electron-dense matrices.

Cells of the inferior part of the leaf were less affected by the methane. In these cells, the chloroplasts had a more circular form and accumulation of starch granules was not noticeable. Some of the mitochondria had a lightened matrix.

The action of ethane on the plant cells differed slightly from the action of methane. In upper part of the maize leaf, normal chloroplasts with grains of a crescent shape were observed. A small part of the chloroplasts was stretched and elongated. Some chloroplasts contained large quantities of starch grains (Fig. 56).

The chloroplasts in the cells of the middle part of the leaf acquired an elongated shape. They contained in most cases starch grains and only the chloroplast matrix became noticeable. Swollen membranes surrounded the chloroplasts. Chloroplasts in various stages of destruction were found: with destroyed external membranes, and with dissociated grains in the thylakoids. In these cells the mitochondria were invaginated, had electron-dense matriceswith widened cristae. The cell cytoplasm was lightened (Buadze et al., 1979). In lower part of the leaf, ethane did not cause any specific deviations of the the shape and structure of the chloroplast, similar to methane. The chloroplasts had clearly visible grains and thylakoids, and the mitochondria had dense matrices with swollen cristae.

The effect of another gaseous alkane, i.e., **propane, butane,** and mixtures of alkanes with composition similar to **natural gas** (methane 88.7%, ethane 6.8%, propane 2.8% and butane 1.7%) in dynamics have been investigated (Buadze et al., 1979; Buadze, Kvesitadze, 1997).

Thus, generalizing the above indicated results, it’s evident that chloroplasts of upper and middle parts of the leaf are more sensitive to the action of gaseous alkanes (Fig. 57, 58). Densitometry analysis of chloroplast tylakoids shows that after 24 h of incubation of leaves in the atmosphere of alkanes average area of chloroplasts increases from methane to butane, and on the contrary, thickness and distance between membranes of thylakoids decrease.

It seems that, one of the main pathological effects, characteristic of low-molecular alkanes is the ability to swell chloroplasts and cause morphological and ultrastructural changes at a membrane level. Swelling of thylakoid membranes of chloroplasts, inducing their morphological change, leads to the significant decrease of matrix of chloroplasts which finally negatively affects the normal functioning of biochemical process, proceeding in a plant photosynthetic apparatus.

After cultivation of pea seedlings in the atmosphere, containing **propylene** and **butylene** (ratio with air 1:4, duration of exposure 72 h), in the upper part of the leaf toxicant action is manifested on plastids and mitochondria, in which partial plasmolysis is observed. Unlike the alkanes in this case reduction of chloroplast sizes, destruction of lamellar systems and accumulation of starch grains take place. Mitochondria are swollen their matrix become dense cristae are widened. In cells of the middle part of the leaf destructive action of propylene is enhanced. Particularly, more intensive accumulation of starch is seen in plastids, in some cells chloroplasts are destructed and disseminated in cytoplasm some separate grains and thylakoids are observed. Mitochondria are at the initial stage of destruction – the lost of matrix content makes them lightened, large quantities of cristae disappear, leaving empty shape of double membrane. Inferior part of the leaf undergoes the great changes, mainly at a level of chloroplasts and mitochondria. Cytoplasm becomes lightened, nucleus friable, membrane complex is badly seen.

Ensuing from the above mentioned data it can be concluded, that low alkenes induce somehow similar changes of cell structure. Unlike the destructive effects of alkanes on the upper and middle parts of the leaf, alkenes more affect the inferior parts of the leaf. In both cases chloroplasts and mitochondria are more damaged.

Action of Organic Contaminants Containing an Aromatic Ring

As a result of [1-6-^{14}C] **benzene** action on cell ultrastructural organization of perennial wood plant leaves, first of all pathological changes are observed in a photosynthetic apparatus. Particularly this is expressed in disorganization of a complex chloroplast-lamellas-grains and appearance of osmiophilic insertions in chloroplasts (Fig. 59). According to these features, i.e. according to the resistance of the photosynthetic apparatus to benzene action, plants highly resistant to benzene action such as lime, maple, silver fir (*Abies*), poplar, Norway spruce, common fir (*Picae*), nut tree (*Iuglans regia*), platan (*Platanus*), cypress (*Cupressus*), ash tree (*Fraxinus excelsior*) have been revealed.

Effect of small concentration of benzene vapors ($4\cdot10^{-5}$ M) on cell ultrastructure of leaves of 7 day old bean seedlings causes only insignificant changes, expressed in manifestation of lightened fragments in chloroplast matrix. At five fold increase of benzene concentration ($2\cdot10^{-4}$ M) its destructive effect is revealed only in chloroplasts, expressed in disorientation of lamellas and thylakoids. Total cell destruction occurs at $4\cdot10^{-4}$ M concentration of benzene in chloroplasts intramembrane system and matrix are

destructed, cell wall is thickened, in periplasma myelin insertions are observed, and in vacuoles – osmiophilic insertions, mitochondria are electron dense.

Aromatic nitroderivatives ***p*-nitrobenzene, *o*-nitrophenol** and **2,4-dinitrophenol** (at concentrations 10^{-3} M) induce total destruction of the cell ultrastructure in the upperr and lower parts of leaves. Benzene, **phenol**, *o*-nitrophenol and ***o*-cresol** induce pathological destructions only in the lower part of leaves. Probably different toxicity of xenobiotics is stipulated not only by presence of substantial functional groups, but also by their location in molecule (Meskhi at al., 1973).

After penetration of polycyclic hydrocarbons (**benz[a]antracen** and **3,4-benzpyrene**) and aromatic amine **benzidine** through roots, the first signs of destruction of the cell ultrastructure are revealed in nuclei (Fig. 60): the configuration of nuclear membrane is significantly changed, nucleus becomes invaginated. Small concentrations (10^{-4}M) of 3,4-benzpyrene don't have sharply expressed pathological effect on cell structure. Supposedly, due to their deep oxidative transformations, the degradation products insert into the metabolic processes by further oxidation to carbon dioxide (Korte et al., 2000).

Namely, these concentrations of toxicants can be considered as metabolic concentrations. At further enhancement of concentration (up to 10^{-3} M) the chromatin coagulation of different forms and sizes are observed, which points to the damage of DNA synthesis. Mitochondria loose internal content and become light. Afterwards, at concentration 10^{-2}M cell total destruction starts. It should be mentioned, that plastids appeared more resistant to polycyclic aromatic hydrocarbons.

Another example of pathological action dependence on toxicants rising concentrations is the action of herbicides **dinitro-*o*-cresole** (DNOC) and 2,4-D on the photosynthetic apparatus of cells of vine leaves.

This herbicide causes the loss of normal outline of the surface of vine leaves. In cells of such leaves smoothing of mitochondrial crista is observed. Another characteristic deviation from the norm is expressed in reduction of sizes of stomata. Such structural changes of epidermis finally lead to the loss of leaf elasticity (Buadze, Kvesitadze, 1997).

In cells of soybean roots, incubated for 5 days with $5 \cdot 10^{-4}$M solution of labeled [1-^{14}C] **TNT**, penetrated into the cell toxicant is manifested as an electron-dense label in cell walls, endoplasmic reticule, mitochondria, plastids, nucleolus and vacuoles (Fig. 61) The given concentration of TNT induces the total cell destruction of maize roots. In soybean leaves the labeled TNT is detected in cell wall, chloroplasts and vacuoles. In cells of maize leaves, distribution of labels is similar. Comparative analysis creates basis to conclude that: soybean is more resistant to TNT action as compared with maize (Unpublished data of authors).

Attention should be paid to the localization of TNT on membrane structures, participating in the transport of reducing equivalents (membranes of endoplasmic

reticulum, mitochondria, and plastids). These membranes are contacting each other in many places. Supposedly, TNT transformation, accomplished in plant cell for detoxification of this contaminant proceeds in these places.

Ultrastructural Reorganization of the Plant Cell During Detoxification

On shown in the previous chapter, it is obvious that plants significantly differ significantly in their ability to assimilate organic toxicants that if penetrated into the cytoplasm, are incorporated with different intensity into the subcellular organelles. For the first 30 min toxic compounds penetrate and accumulate in the subcellular organelles. Simultaneously, the induction of specific enzymes takes place that participate in by further oxidizing transformations of the toxicants (Khatisashvili et al., 1997; Kvesitadze et al., 2001). All toxic compounds investigated changed the plant cell structure to a different extent. Despite the fact, that at lower, so-called metabolic concentrations, the normalcytological picture did not change, it should be taken into consideration that even in this case some deviations in cell ultrastructure (e.g. widening of periplasmic space, reducing plasmodesmata, increase in the volume of endoplasmic reticulum, etc.) take place.

Attention should be paid to the processes promoting the detoxification of toxic compounds and their removal from the cell. Among such processes, the deposition in the vacuole of xenobiotics that penetrated into the cell must be emphasized. This phenomenon, observed practically in all cases where labeled organic toxicants were used, allows the cell to resist the destructive action of the toxicantand send it to the vacuole excluding it from interfering with normal cell metabolism

Partially transformed toxicants are disposed in the vacuole. These are conjugates of xenobiotics and/or intermediate products of their transformation with intracellular compounds (proteins, peptides, low-molecular sugars etc.). For instance, after penetration of labeled 2,4-D into the root cells of barley seedlings, conjugates were detected in the vacuoles, and among these conjugates 80% were O-β-D-glycosides of the herbicide metabolites (Chkanikov, 1985).

Usually, the quantity and size of vacuoles are significantly increased by the action of the toxicant. In addition to the intensification of vacuolization, the splitting of some small vacuoles and formation of larger organelles, occupying most of the cell are often visible.However, , as soon as the cell is given a chance, the process of removing the toxic residues from the vacuoles to the extracellular and subsequently to the intercellular spaces begins. This phenomenon is observed after terminating the exposure of the plant In such cases the periplasmatic space of the cell is appreciably widened. The agranulation of the rough endoplasmic reticulum begins, then cysterns of smooth endoplazmic reticulum connect with vacuoles through which part of the conjugates are excreted from cell. Besides the fragmentation of the endoplasmic reticulum cisterns takes place, which proceeds in the form of vesicles that are translocated to the cell periphery. In the final phase of the process of exocytose, secretion process is activated. The

formation of multiple contacts of vesicles with the plasmalemma confirms this hypothesis. Fusion of the membranes of vesicles and plasmalemma proceeds via the participation of Ca^{2+}-binding centers. As a result, the vesicle contents (conjugates of toxicants) are removed to the periplasmatic space (Fig.62 b,c,d) (Unpublished data of the authors).

Fragments of the smooth endoplasmic reticulum participate in another simpler process of secretion of vesicle contents beyond the cell. In this case, the channels connecting the vacuoles with the plasmalema for the translocation of the toxic remains from the vacuole to the intercellular space are formed by means of the smooth membrane fragments (Fig. 62 a) (Unpublished data of the authors).

Often a large number of ribosomes is visible in the plant cells under the influence of a toxic compound. This phenomenon points to an increase in protein biosynthesis. The electron microscopic analyses of ultra-thin cuttings of soybean and maize roots apices, under the influence of nitrobenzene in different concentrations, clearly showed the appearance of cells, darkened by multiple ribosomes (Zaalishvili et al., 2000). At lower concentrations of nitrobenzene ($1.5x10^{-5}$) such cells were found only in the apex of maize roots. In soybean cells, rich in reserve protein complexes, the numberof ribosomes increased only at a concentration of $1.5x10^{-3}$ M, when a store of proteins was consumed. The increase in protein biosynthesis must promote on one hand the reduction in theamount of protein used during conjugation with the toxic compounds and their metabolites, and on the other hand, the induction of enzymes participating in the detoxification. Histochemical and biochemical analyses showed that at this time, the induction of enzymes important for detoxification (peroxidases, cytochrome P450 - containing monooxygenases, phenoloxidases) take place. The content of these oxidizers is significantly enhanced in the cell wall, on membranes of the plasmalema, endoplasmic reticulum, tonoplasts and vacuoles, i.e. where suitable conditions are created to detoxify the toxicant far removed from the informational and energetic centers of the cell (Kvesitadze et al., 2001).

Cells attempt to minimize the destructive action of xenobiotics via their deep degradation, and this is expressed not only in the induction of detoxificationenzymes, but also in the creation of optimal conditions for their effective functioning. Organic toxicants, that undergo oxidative and reductive transformations, often induce multiple contacts between organelles (mitochondria, endoplasmic reticulum, and plastids), having a redox chain for electron transport on membranes. For instance, after exposure of root cells to nitrobenzene, the contacts between the endoplasmic reticulum and the mitochondria were quantified and it turned out that . many mitochondria were surrounded by endoplasmic membranes (Fig. 63). Such ultrastructural reorganization allows the mitochondrial and microsomal electron-transporting systems to provide reducing equivalents to thecytochrome P450- containing monooxygenase system located on endoplasmic membranes. Cytochrome P450 uses these electrons for the activation of molecular oxygen and the hydroxylation of xenobiotics, which is the rate limiting stage of the whole detoxification process. It is interesting to note that such phenomenon is

observed both in animal (Schenckman et al., 1973) and in plant (Gordeziani et al., 1999) cells, and is known as the "mitochondrial control" on xenobiotic oxidation.

Similar ultrastructural changes leading to the contacts of membrane structures are observed during TNT exposure of plant cells. In this case, other providers of reducing equivalents, i.e. plastids, are in contact with the endoplasmic reticulum, together with mitochondria. In this case, electrons are needed not for oxidation, but for reduction of the nitro-groups of the toxicant, leading to the formation of the less toxic amino- derivatives of toluene.

Thus, in the plant cell the coordination of the energetic and plastic metabolism takes place and the energy, needed for detoxification, is supplied by the corresponding enzymes in the form of electrons, not in the form of ATP. By this mechanism the cell mobilizes redox enzymes and inserts them in the detoxification of toxic compounds.

11 - CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Recently, phytoremediation became an important and commercially viable biotechnology to clean up the environment. This technology is based on the ability of certain plant species to absorb and degrade organic pollutants from soil and water and accumulate inorganic contaminants in root system and aboveground parts of the plants. Various types of phytoremediation such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization, phytovolatalization solely by plants or in combination with specially selected single, groups of microorganisms, or microbial consortia, due to their high effectiveness, are transferred from pilot projects into commercial scale operations, establishing credibility of this technology. The progress with phytoremediaton of organics has been far greater and more rapid than of inorganics and radionuclides. The level of success and future of this technology is entirely dependent upon the selection of appropriate plant and microorganism species, and exploitable industrial characteristics, physiology, morphology and adaptability to agronomic practice. The efficiency of phytoremediation as well as management of contaminated landscapes is carried out by plants and microbial consortia directed to the degradation of contaminants of different chemical structure. It also can be recognized that for decades plant uptake can be exploited as a biological clean-up technology for inorganic contaminants. Therefore, phytoextraction is a low-cost *in situ* "green" technology for the clean-up of metal polluted soils. This technology offers the possibility of selectively removing only the metal contaminants from soil. Phytoextraxtion is seen as an alternative technology for progressive long term remediation or sustainable management of soils where the majority of metals are stay in the rhizossphere.

Cleanup of decontaminated soils, ground waters and water reservoirs with high concentrations of contaminants become a problem of worldwide environmental priority. In addition to different small-scale artificial technologies (chemical, mechanical), which have already bean developed for cleanup of polluted areas, comparatively new, natural technologies based on the use of soil microorganisms and plants, have also been demonstrated over the past two decades.

Plants, microbes and soil interactions, and their relationships are interdependent and factors determining the effectiveness of the remediation. Soil is a complex system made up of a heterogenous mixture of solid, liquid, and gaseous components. While soil provides the supporting medium, plants build up the organic matter content in the soil and support the multiplication and action of the microbial community by releasing root exudates. The soil microbial community in its turn degrades (by oxidation or hydrolysis) compounds that are diffcicult to absorb by plants to easily transportable and metabolizable substances. Such cooperation between plant and soil microbial consortium has a clearly expressed symbiotic character and significantly accelerates all plant metabolic processes, including the transformation of contaminants. For an industrial application, it would be extremely important to select the proper combination of microorganisms of all taxonomic groups having increased contaminants (for each particular case) degradation ability in combination with plants also having increased tolerance to the action of the same contaminants or their toxic intermediates. Such

individual approaches to each contaminated site would most effectively decrease the level of contamination and increase the fertility of polluted soil. There are also other factors influencing (sometimes significantly) the remediation process. To such factors could be related the treatment of plants and soil by different biologically active preparations: amino acids, organic acids and carbohydrates, such as for example, products of INAGROSA (Spain) effective in activating both plants and microbial metabolic and corresponding remediation processes.

Bioremediation is a complicated process depending on many factors such as: plant variety, activity of microorganisms, fertility of the soil, type of contaminant, temperature, amount of oxygen in soil, etc. That is why it is difficult to determine the real input of each factor in the bioremediation process, but itis absolutely clear that in plants as well as in microbes intracellular degradation of contaminants in the great majority of the cases are performed by oxidative enzymes, namely cytochrome P450 dependent monooxygenase, peroxidase and phenoloxidase in plants and Mn-dependent peroxidase and laccase in microorganisms. Despite that in the process of bioremediation quite a few enzymes participate, we don't have a clear understanding of the mechanisms of their action, interchange and substrate specificity, which is very important. There is no doubt that the rate of the remediation process is limited by one or more of theabove-mentioned enzyme activities. To overcome the limitations of the remediation process, it should be revealed which enzymatic stage is important for the creation of new genetically modified effective transformedplants and microorganisms. It is well-known, that over the past decade quite a few genetic engineering approaches have been carried out to improve the plants efficiency to remeove contaminants. For this reason, it is extremely important to know which enzymatic reactions (oxidases, reductases or transferases) are process-rate limiting factors, and to double the genes of those particular enzymes in created transforms. The degradation process of some contaminants is significantly complicated, for instance the degradation by plants of such explosives as TNT is complicated, because of involvement of both oxidative and reductive enzymes. This process requires additional information concerning the participation of all enzymes, as well as an investigation of the consequences of enzymatic reactions. This is the main gap of our not complete understanding of the detoxification process.

Bioremediation is a multistage process, combining the use of plants and microorganisms in landscape management. It implies different approaches based on multiple factors beginning from plant and microbial physiology and ending by genetic engineering.

For the successful remediation of a polluted environment it is extremely important to know all aspects determining the bioremediation potential and among them the most important are enzymatic mechanisms of detoxification of selected organic contaminants both in higher plants and in microorganisms.

An important step in this direction is international recognition of the problem and cooperation of multi-expertise scientific centers in different issues related to phyto/bioremediation, including:

1. To continue the selection of new microbial strains degrading organic contaminants, including explosives and plants characterizing by high tolerance to the same contaminants.
2. To reveal and characterize the new and known enzymes participating in both microbial and plant degradation processes.

In the Appendices A and B lists are presented of plant and microbial strains potentially utilizable for remediation purposes.

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Appendix A- Plant Species that Might be Utilized for the Remediation of Organic Contaminants

Organic Contaminant	Plant Species	Comment	Reference
1	2	3	4
Aromatic hydrocarbons (benzene, toluene)	Maple (*Acer campestre*) Oleaster (*Elaeagnus angustifolia*) Locust (*Robinia pseudoacacia*) Wild pear (*Pyrus caucasica*) Walnut *(Juglans regia)* Almond (*Amigdalus communis*) Cherry (*Cerasus avium*) Cherry (*Cerasus vulgaris*) Amorpha (*Amorpha fructicosa*) Chestnut (*Castanea sativa*) Apple (*Malus domestica*) Zelkova(*Zelcova caprinifolia*) Poplar (*Populus canadensis*) Ryegrass (*Lolium perenne*) Lilac (*Siringa vulgaris*) Weeping willow (*Salix*) Catalpa (*Catalpa bignonioides*) Platan (*Platanus orientalis*) Sophora (*Sophora japonica*)	Plants capable to absorb 1-10 mg of benzene and toluene per kg fresh leaves per day from air	Ugrekhelidze, Durmishidze, 1980; Durmishidze, 1988; Unpublished data of authors
	Alfalfa (*Medicago sativa* L.)	Plants remove benzene from soil	Ferro et al., 1997
		Plants enhance biodegradation of toluene by associated microorganisms	Davis et al., 1994
Gaseous alkanes (methane, ethane, propane, butane)	Tea (*Thea sinensis*) Vine (*Vitis vinifera*) Poplar (*Populus canadensis*) Walnut *(Juglans regia)* Maple (*Acer campestre*) Ryegrass (*Lolium multiflorum*) Maize (*Zea mais)* Kidney bean (*Phaseolus vulgaris*)	Plants are capable to absorpb 0.1-10 mg of gaseous alkanes per kg fresh leaves per day from air	Unpublished data of authors

1	2	3	4
Petroleum hydrocarbons (PHC)	Pine (*Pinus sylvestris* L.)	Plant roots enhance rhizospheric degradation of PHC in soil	Heinonsalo et al., 2000
	Alfalfa (*Medicago sativa* L.)	Plants remediate crude-oil contaminated soil	Wiltse et al., 1998
	Spartina alterniflora (salt marsh species) *Juncus roemerianus* (salt marsh species) *Spartina patens* (brackish marsh species) *Sagittaria lancifolia* (freshw. marsh species)	Plants remediate oil spills in marshes	Lin, Mendelssohn, 1997
	Clover *(Trifolium)* Tall fescue (*Festuca arundinacea* Schreber) Bermuda grass *(Cynodon dactylon)* Ryegrass (*Lolium multiflorum*)		Schwab et al., 1998
	Ryegrass (*Lolium perenne*)	Plants remediate PHC-contaminated soil and dredged material	Hou et al., 2001 Best et al., 2004
PAHs	Ryegrass *(Lolium multiflorum)* Hybrid poplar (*Populus* sp.) Clover (*Trifolium* spp.)		Susarla et al., 2002
	Tall fescue (*Festuca arundinacea* Schreber) Alfalfa (*Medicago sativa* L.)	Terrestrial plants capable to absorb and degrade naphtalene	Schwab et al., 1998
	Sorghum (*Sorghum bicolor)* Switchgrass (*Panicum virgatum)*	Plants enhance rhizospheric degradation of PAHs in soil	Reilley et al., 1996
	Prairie buffalograss (*Buchloe dactyloides* var. 'Prairie') Kleingrass (*Panicum coloratum* var. 'Verde')	Decrease of naphtalene content in clay soil	Qiu et al., 1997
	Big bluestem (*Andropogon gerardii)* Little bluestem (*Schizachyrium scoparius)* Indiangrass (*Sorghastrum nutans)* Switchgrass (*Panicum virgatum)* Canada wild rye (*Elymus canadensis)* Western wheatgrass (*Agropyron smithii)* Side oats grama (*Bouteloua curtipendula)* Blue grama (*Bouteloua gracilis)*	A mixture of prairie grasses that degrade PAHs	Aprill, Sims, 1990

1	2	3	4
Phenols	Horseradish (*Armoracia rusticana* P. Gaerter, Meyer & Schreb*)* Potato (*Solanum tuberosum)* White radish *(Raphanus sativus)*	Plants have a highly active peroxidase that oxidizes phenols (used in wastewater treatment)	Dec, Bollag, 1994
	Soybean (*Glycine max* L. Merr. Cv. Fiskby v)		Fletcher et al., 1990
	Reed (*Scirpus lacustris* L.)		Seidel, Kickuth, 1967
	Alfalfa (*Medicago sativa* L.)	Plant enhances degradation of phenol by associated microorganisms	Davis et al., 1994
Polichlorinated solvents Trichloroethane (TCE) Tetrachloroethane	Hybrid poplar (*Populus trichocarpa x P. deltoides),* Aspen (*Populus* sp.) Cottonwood (*Populus* sp.)	Poplars transpired, metabolized or mineralized 98% of TCE in soil at a concentration of 260 mg kg^{-1}	Kassel et al., 2002
	Soil green alga *Chlamidomonas reinhardtii* Marine green alga *Dunaliella tertiolecta*	Algae able to absorb and degrade TCE at a concentration of 500 mg kg^{-1}	Dresback et al.,2001
	Wild carrot *(Daucus carota)* Spinach *(Spinacia oleracea)* Tomato (*Lycopersicon esculentum)*	Plants absorb and transform TCE from groundwater	Schnabel et al., 1997
	Waterweed (*Eichhhornia crassipes)*		Roy, Hanninen, 1994
	Black locust (*Robinia pseudoacacia)*	Plants volatilize TCE from groundwater	Newman et al., 1999
	Hybrid poplar (*Populus* sp.)		Burken, Schnoor, 1997
	Alfalfa *(Medicago sativa)*	Plants exudates promote degradation of TCE in its rhizosphere	Narayanan et al., 1999
Dibromoethane TCE	Koa haloe (*Leuceana leucocephala* var. K636)	Tropical leguminous tree species	Doty et al., 2003
TCE	Lespedeza (*Lespedeza cuneatea* (Dumont)) Loblolly pine (*Pinus taeda* L.) Soybean (*Glycine max* L. Merr. Cv. Davis)	Plants cause increased mineralization of TCE in soil	Anderson, Walton, 1995
Chlorinated benzoic acid	Slender wheatgrass (*Agropiron pinnata)* Westerm wheatgrass (*Agropiron smithii)*	Prairie grass species	Siciliano, Germida, 1998 Topp et al., 1989
Nitrobenzene	Soybean (*Glycine max* L. Merr. cv. Fiskby v)		Fletcher et al., 1990

1	2	3	4
PCBs	Red mulbery (*Morus Rubra* L.) Crabapple (*Malus fusca (*Raf.) Schneid) Osage orange (*Maclura pomifera* (Raf.) Schneid)	Plants produce exudates that stimulate growth of PCB-degrading bacteria	Fletcher, Hedge, 1995
	Spearmint (*Mentha spicata)*	Plant that induces cometabolism of PCB in its' rhizosphere	Gilbert, Crowley, 1997
	Barley (*Hordeum vulgare* L. cv.Klages)		McFarlane et al., 1987
Delor 103	*Solanum nigrum* L	Plant causes 40% mineralization of 100 mg PCB kg^{-1} soil in 30 days	Mackova et al., 1997
Arochlor 1260	Tall fescue (*Festuca arundinacea* Schreb.) Alfalfa (*Medicago sativa* L) Flatpea (*Lathyrus sylvestris* L.) Sericea lespedeza (*Lespedeza cuneata* Dum) Deertongue (*Panicum clandestinum* L.)	Plants enhance mineralization in soil microcosm; decrease levels from 100 to 23-33 mg PCB kg^{-1} soil in 4 months	Epuri, Sorensen, 1997
Pesticides Simazine [2-chloro-4,6-bis(ethylamino)-1,3,5-triazine]	Parrot feather (*Myriophyllum aquaticum* (Vell.) Verdc.) Canna (*Canna* x *hybrida* L. 'Yellow King Humbert').		Knuteson et al., 2002
Hexachlorobenzene Pentachlorobenzene Trichlorobenzene	Barley (*Hordeum vulgare* L. cv.Klages)		McFarlane et al., 1987
Atrazine	Hybrid poplar (*Populus deltoides x nigra* DN34 Imperial Carolina.)		Burken, Schnoor, 1997
	Kochia (*Kochia scoparia* L.Schrad)	Plant enhances rhizospheric mineralization of atrazine in soil	Perkovich et al., 1996
	Pine *(Pinus ponderosa)*	Plant supports degradation by ectomycorrhizal fungus *Hebeloma crustuliniforme*	Gaskin, Fletcher, 1997
Chloroacetamides	Maize (*Zea mays* L.)		Hoagland et al., 1997
Metolachlor with Atrazine	Coontail *(Ceratophyllum demersum),* Canadian pondweed *(Elodea canadensis)* Common duckweed *(Lemna minor)*	Aquatic plants remediate herbicides in water	Rice et al., 1997

1	2	3	4
Pesticides-ctd Chlorinated Phenols (4-Chloro-phenol to penta-chlorophenol)	Duckweed (*Lemna gibba)*	Floating plant remediates herbicides in water	Ensley et al., 1997
Cyanazine with Fluometuron	Ryegrass *(Lolium multiforum* L.) Hairy vetch *(Vicia villosa* Roth*)* Rice *(Oryza sativa* L.)	Plants enhance the degradation of herbicides in soil via stimulation of bacterial populations and enzyme activities	Wagner, Zablotowicz, 1997
Pentachlorophenol (PCP)	Wheat grass (*Agropyron cristatum)*	Plant roots enhance rhizospheric degradation of PCP in soil	Ferro et al., 1994
	Hard fescue (*Festuca ovina* var. *duriuscula)* Tall fescue (*Festuca ovina* var. *duriuscula)* Red fescue (*Festuca ovina* var. *duriuscula)*	A mixture of fescues with high germination rates and high biomass in PCP- and PAH-contaminated soil	Pivetz et al., 1997
	Waterweed (*Eichhornia crassipes)*		Roy, Hanninen, 1994
	Crested wheatgrass (*Agropyron desertorum* Fischer ex Link Schultes)	Plant enhances mineralization of PCP to 23.1 mg kg^{-1} soil in 20 weeks	Ferro et al., 1994
Parathion Diazinon	Bush bean (*Phaseolus vulgaris* cv. 'Tender Green')	Plants enhance rhizospheric degradation of herbicides	Hsu, Bartha, 1979
Bentazon	Black Willow *(Salix alba)* Bald cypress (*Taxodium distichum)* River bitch *(Betula nigra)* Cherry-bark oak *(Quercus falcata)*	Plants have a high capacity to degrade bentazon	Conger, Portier, 1997
	Live oak *(Quercus viginiaga)*		
Aldrin Dieldrin	Arctic hairgrass (*Deschampsia bernigensis*) Felt leaf willow (*Salix alaxensis)* Red fescue (*Festuca rubra*) Spikerush *(Eleocharis palustris)*		Williams et al., 2000
2,4-D DDT	Hybrid poplar (*Populus* sp.)		Torres et al., 1999

1	2	3	4
Pesticides-ctd Atrazine Metolachlor Trifluralin	Kochia (*Kochia scoparia* L.Schrad) Knotweed (*Oiktgibyn* sp.) Crabgrass (*Digitaria* sp.*)*	Plants enhance microbial degradation in rhizosphere, i.e., 45% of atrazine, 50% of metolachlor and 70% of trifluralin in 14 days	Anderson et al., 1994
Explosives-ctd 2,4,6-Trinitro-toluene (TNT)	Parrot feather (*Myriophyllum aquaticum)* Water milfoil *(Myriophyllum spicatum)*	Aquatic plants	Pavlostathis et al., 1998 Vanderford et al., 1997
	Stonewort (*Nitella*)	Algae	
	Parrot feather (*Myriophyllum aquaticum*) Sweet-flag (*Acorus calamus* L.) Wool-grass (*Scirpus cyperinus* L. Kunth) Waterweed (*Elodea canadensis* Rich. in Michx) Sago pondweed (*Potamogeton pectinatus* L.) Water star-grass (*Heteranthera dubia* Jacq. MacM) Curlyleaf pondweed (*Potamogeton crispus* L.)	Emergent and submersed plant species with a high ability to remove TNT from water, and recommended for phytoremediation of explosives-contaminated water on Army Ammunition Plants	Best et al.,1999a, b
	Switchgrass (*Panicum virgatum)*	Prairie grass species	Peterson et al., 1998
	Bush bean (*Phaseolus vulgaris* cv. 'Tender Green')		Harvey et al., 1990
	Hybrid poplar (*Populus* sp.)		Burken, Schnoor, 1997; Thompson et al., 1998
	Soybean (*Glycine max*) Ryegrass *(Lolium multiflorum)* Pea (*Pisum sativum*) Chickpea (*Cicer arietinum)*	Plants that absorb 0.15-0.20 mg TNT per gram fresh biomass per day	Unpublished data of authors
	Bromegrass (*Bromus erectus*)	Plant-microbial consortium with *Pseudomonas* sp.	Siciliano et al., 2000
	Spinach (*Spinacia oleracea*)		Medina et al., 2002

1	2	3	4
Explosives-ctd Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	Parrot feather (*Myriophyllum aquaticum* Vell. Verdc.) Sweet-flag (*Acorus calamus* L.) Wool-grass (*Scirpus cyperinus* L. Kunth) Waterweed (*Elodea canadensis* Rich. in Michx) Sago pondweed (*Potamogeton pectinatus* L.) Water star-grass (*Heteranthera dubia* Jacq. MacM) Curlyleaf pondweed (*Potamogeton crispus* L.)		Best et al., 1999a, b
	Parrot feather (*Myriophyllym aquaticum*) Spinach (*Spinacia oleracea*) Mustard (*Brassica juncea)*		Medina et al., 2002
	Hybrid poplar (*Populus deltoides x nigra*, DN34)		Yoon et al., 2002
	Bush bean (*Phaseolus vulgaris*)		Harvey et al., 1990
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	Alfalfa (*Medicago sativa*) Bush bean (*Phaseolus vulgaris*) Canola (*Brassica napa*) Wheat (*Triticum aestivum*) Perennial ryegrass (*Lolium perenne*) Wild bergamot (*Monarda fistulosa*) Western wheatgrass (*Agropyron smithii*) Bromegrass (*Bromus sitchensis*) Koeleria (*Koeleria gracilis*) Goldenrod (*Solidago* sp.) Blueberry (*Vaccinium* sp.) Anemone (*Anemone* sp.) Common thistle (*Circium vulgare*) Wax-berry (*Symphoricarpos albus*) Western sage (*Artemisia gnaphalodes*) Drummond's milk vetch (*Astragalus drummondii*)	Terrestrial indigenous and crop plants capable to absorb, translocate and accumulate HMX in foliar tissues (selected from contaminated soil from an anti-tank firing range)	Groom et al., 2002

Appendix B- Microbial Species that Might be Utilized for the Remediation of Organic Contaminants

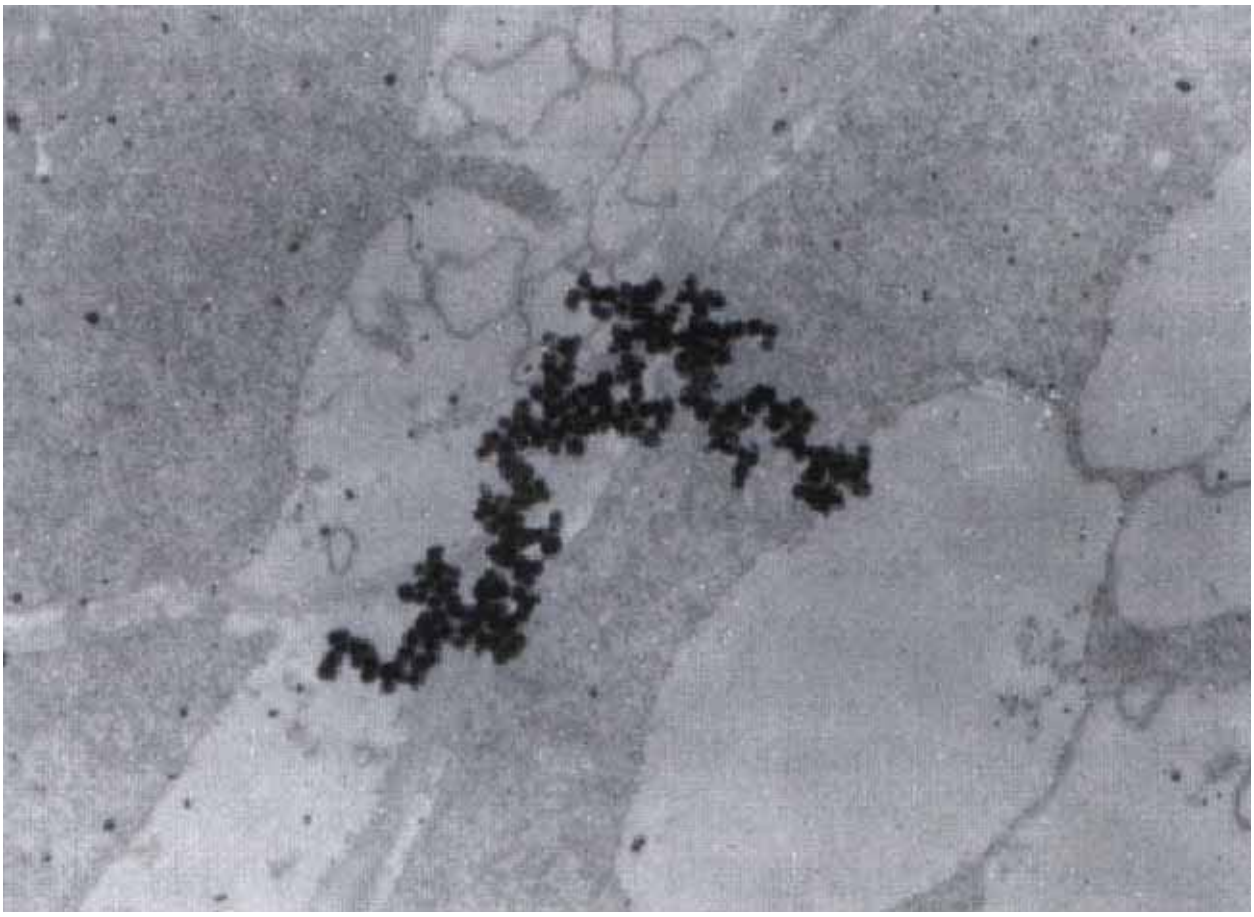
Organic Contaminant	Microbial Species	Comment	Reference
1	2	3	4
Aromatic Hydrocarbons (benzene, toluene, cresols, etc)	*Burkholderia cepacia* Strain G4 PR1	Strains have active toluene ortho-monooxygenase	Snyder, 1998
	Pseudomonas cepacia Strain G4		Folsom et al., 1990
	Pseudomonas cepacia Strain G4 PR1		Shields et al.,1993
	Pseudomonas cepacia *Pseudomonas putida* *Pseudomonas mendocina* *Nocardia* sp. *Alcaligenes* sp. *Acinetobacter* sp.		Shields et al., 1990
Alkanes	*Methylococcus* sp *Methylomonas* sp. *Candida biodinii* *Hansenolla polymorpha*	Strains capable to oxidize methane to CO_2	Kvesitadze, Bezborodov, 2002
	Nocardia sp. *Flavobacterium* sp *Mycobacterium* sp. *Pseudomonas* sp.	Strains capable to oxidize ethane, propane and butane to aliphatic alcohol with further β-oxidation to CO_2	
	Candida lypolitica *Candida tropicalis*	Strains capable to oxidize long-chained carbohydrates (> 15 C-atoms)	
	Pseudomonas cepacia Strain G4 PR1		Shields et al., 1993
Total Petroleum Hydrocarbons (TPH)	*Rhodococcus* sp. Strain 13 *Rhodococcus* sp. Strain 23 *Rhodococcus* sp. Strain 124 *Rhodococcus* sp. Strain 227 *Rhodococcus* sp. Strain 235 *Mycobacterium* sp. Strain 99 *Aspergillus niger* sp. Strain 35 *Trichoderma* sp. Strain 9 *Trichotecium* sp. Strain 65	Strains capable to assimilate >80% oil from soil at a concentration of 30 g kg^{-1}	Unpublished data of authors

1	2	3	4
PAHs	*Phanerochaete chrisosporium*		Tatarko, Bumpus, 1993 Bogan, Lamar, 1995
	Trametes versicolor	Strain has laccase that oxidizes 3,4-benzoapyrene, acenaphthene, acenaphthylene, anthracene and fluorene	Collins et al., 1996 Johannes, Majcherczyk, 2000
	Sphingomonas paucimobili Strain EPA505	Strain degrades fluoranthene	Trust et al., 1995
	Pseudomonas sp. Strain A2279	Strain degrades acenaphthene	Selifonov et al., 1998
	Pseudomonas paucimobilis Strain EPA 505 *Pseudomonas* sp. Strain SR 3	Strains degrade 51.0% of PAHs in15 days	Middaugh et al., 1994
Phenols	*Pseudomonas cepacia* Strain G4	Strain has active toluene ortho-monooxygenase	Folsom, Chapman, 1991
	Pseudomonas cepacia Strain G4 PR1		Shields et al., 1993
	Burkholderia cepacia Strain G4		Fries et al., 1997
Polychlorinated solvent Trichloroethylene (TCE)	*Burkholderia cepacia* Strain G4 5223-PR1		Winkler et al., 1995
	Burkholderia cepacia Strain G4 *Burkholderia cepacia* Strain PR1301		McCarty et al., 1998
	Pseudomonas cepacia Strain G4	Strain with a capacity to degrade TCE of about 1.1 g d^{-1} g^{-1} cell-protein	Folsom, Chapman, 1991
	Pseudomonas cepacia *Pseudomonas putida* *Pseudomonas mendocina* *Nocardia* sp. *Alcaligenes* sp. *Acinetobacter* sp.	Strains have active toluene ortho-monooxygenase, that participates in degradation of TCE	Shields et al., 1990
PCBs (Aroclors 1242, 1254, 1260)	*Phanerochaete chrisosporium*		Thomas et al., 1992; Yadav et al., 1995
	Arthrobacter sp. Strain B1b		Gilbert, Crowley, 1997

1	2	3	4
PCBs-ctd (Aroclors 1242, 1254, 1260)	*Bacillus* sp. Strain 26 *Bacillus* sp. Strain 33 *Bacillus* sp. Strain 505 *Bacillus* sp. 510	Strains degrade SOVOL (Arochlor-analogue)	Kim et al., 2004
	Pseudomonas sp. Strain LB400	Strain degrades Arochlor 1242, 15% per day, and 50% in 3 days.	Morris, Pritchard, 1994
Pesticides	*Pseudomonas cepacia* Strain G4 PR1		Shields et al., 1993
	Pseudomonas fluorescens		Torres et al., 1999
2,4-D	*Phanerochaete chrisosporium*		Yadav, Reddy, 1993
2,4,5-T	*Burkholderia cepacia* Strain AC1100		Hendrickson et al., 1996
Atrazine	*Hebeloma crustuliniforme*	Consortium with pine (*Pinus ponderosa*)	Gaskin, Fletcher, 1997
DDT	*Phanerochaete chrisosporium* *Phanerochaete sordida* *Phellinus werii* *Polyporus versicolor* *Pleurotus ostreatus*	This strain degrades ~50% DDT in 30 days, with 5–14% of DDT mineralized to CO_2	Bumpus, Aust, 1987 Safferman et al., 1955
	Pleurotus luteoablus	This strain degrades 69% of DDT, 3% of which was mineralised in 30 d at 21 ^oC	Osano et al. ,1999
Chlordane Lindane	*Phanerochaete chrisosporium* BKM-F-1767	This strain degrades 9-15% of Chlordane, and 23% of Lindane 23%, with mineralisation to CO_2	Kennedy et al., 1990
2,4-Dichlorophenol	*Phanerochaete chrisosporium*		Valli, Gold, 1991
2,4,5-Trichlorophenol	*Phanerochaete chrisosporium*		Joshi, Gold, 1993
Pentachlorophenol	*Phanerochaete sordida*		Lamar et al., 1993
	Pseudomonas sp.Strain SR3		Resnick, Chapman, 1994

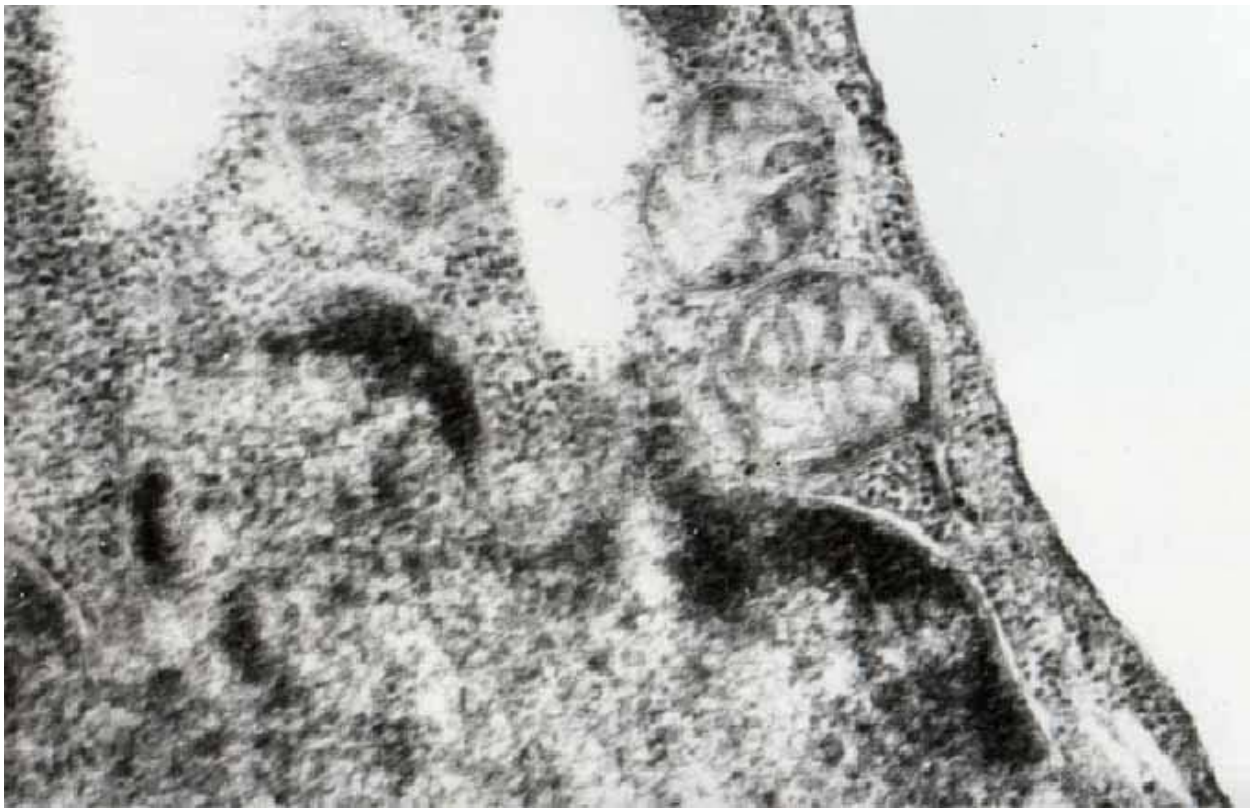
1	2	3	4
Explosives	*Pseudomonas* sp		Haïdour, Ramos, 1996
	Pseudomonas sp.	Plant-microbial consortium with bromegrass (*Bromus erectus*)	Siciliano et al., 2000
	Pseudomonas sp. Strain ST53		Snellinx et al., 2002
	Pseudomonas sp. Strain JLR11	Strain that incorporates 85% of the nitrogen from TNT organic N in cells	Esteve-Núñez et al., 2001
	Pseudomonas sp. Strain C1S1		Duque et al., 1993
	Pseudomonas pseudoalcaligenes Strain JS52	Strain has active nitroreductase	Fiorella, Spain, 1997
	Pseudomonas savastanoi		Martin et al., 1997
	Clostridium bifermentans	Consortium containing *Clostridium spp.*, used for sequential anaerobic remediation of TNT-contaminated soil *ex situ*	Shim, Crawford, 1995;
	Irpex lacteus		Kim, Song, 2001
2,4,6-Trinitrotoluene (TNT)	*Phanerochaete chrisosporium*		Stahl, Aust, 1995
	Phlebia radiata		Van Aken et al., 1999
	Serratia marcescens		Montpas et al., 1997
	Mycobacterium vaccae		Vanderberg et al., 1995
	Rhodococcus sp.VKM Ac 1170 Strain 44 *Rhodococcus* sp. Strain TNT-140 *Rhodococcus* sp. Strain TNT-136 *Rhodococcus* sp. Strain 124 *Genus* sp.*(Micobacterium mucosum* 292) var.Sf *Pseudomonas* sp. Strain TNT-44 *Mycobacterium* sp. Strain 245 *Aspergillus niger* Strain 35 *Mucor* sp. Strain 1 *Trichoderma* sp. Strain 9 *Trichotecium* sp. Strain 65 *Saccharomyces oviformis*	Strains assimilate >80% TNT from contaminated soil at a concentration of 200 mg kg^{-1}	Unpublished data of authors

1	2	3	4
Explosives-ctd	*Serratia macrescens*		Young et al., 1997
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	*Clostridium bifermentans*		Regan, Crawford, 1994
	Rhodococcus sp. Strain A		Jones et al., 1995
	Rhodococcus sp. Strain DN22	Strain uses RDX as a nitrogen source	Coleman et al., 1998
	Rhodococcus sp. Strain YH11		Tekoah, Abeliovich, 1999
	Stenotrophomonas maltophilia Strain PB1		Binks et al., 1995
	Phanerochaete chrisosporium	Strain mineralises 75% of RDX	Fernando, Aust, 1991
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	*Caldicellulosiruptor owensensis*		Huang, 1998
	Alcaligenes sp.	Strains extreme thermophile anaerobes, degrade 30 mg HMX L^{-1} at 75^{o}C	Harkins, 1998
	Hydrogeophaga flava		
	Xanthomonas orizea		
Nitroaromatics Nitrobenzene	*Pseudomonas pseudoalcaligenes*.		Nishino, Spain, 1993
2-Nitrotoluene	*Pseudomonas* sp. Strain JS42		Haigler et al., 1994
4-Nitrotoluene	*Pseudomonas* sp. Strain 4NT		Haigler, Spain, 1993
4-Nitrophenol	*Rhodobacter capsulatus*		Roldán et al., 1998
2,4-Dinitrotoluene 2,6-Dinitrotoluene	*Pseudomonas* sp		Haïdour, Ramos, 1996
Dioxins	*Beijerinckia* sp.		Klecka, Gibson, 1980
	Bacillus sp. *Nocardiopsis* spp.		Matsumura et al., 1983
	Phanerochaete chrisosporium		Bumpus et al., 1985
	Phanerochaete sordida Strain YK-624	Strain degrades 40% tetrachloro-dibenzo-*p*-dioxin; 76% hexachloro-dibenzo-*p*-dioxin; 45% tetrachloro-dibenzofuran; 70% hexachloro-dibenzofuran (initial concentration of dioxins in nutrient medium – 50 pg/ml)	Takada et al., 1996



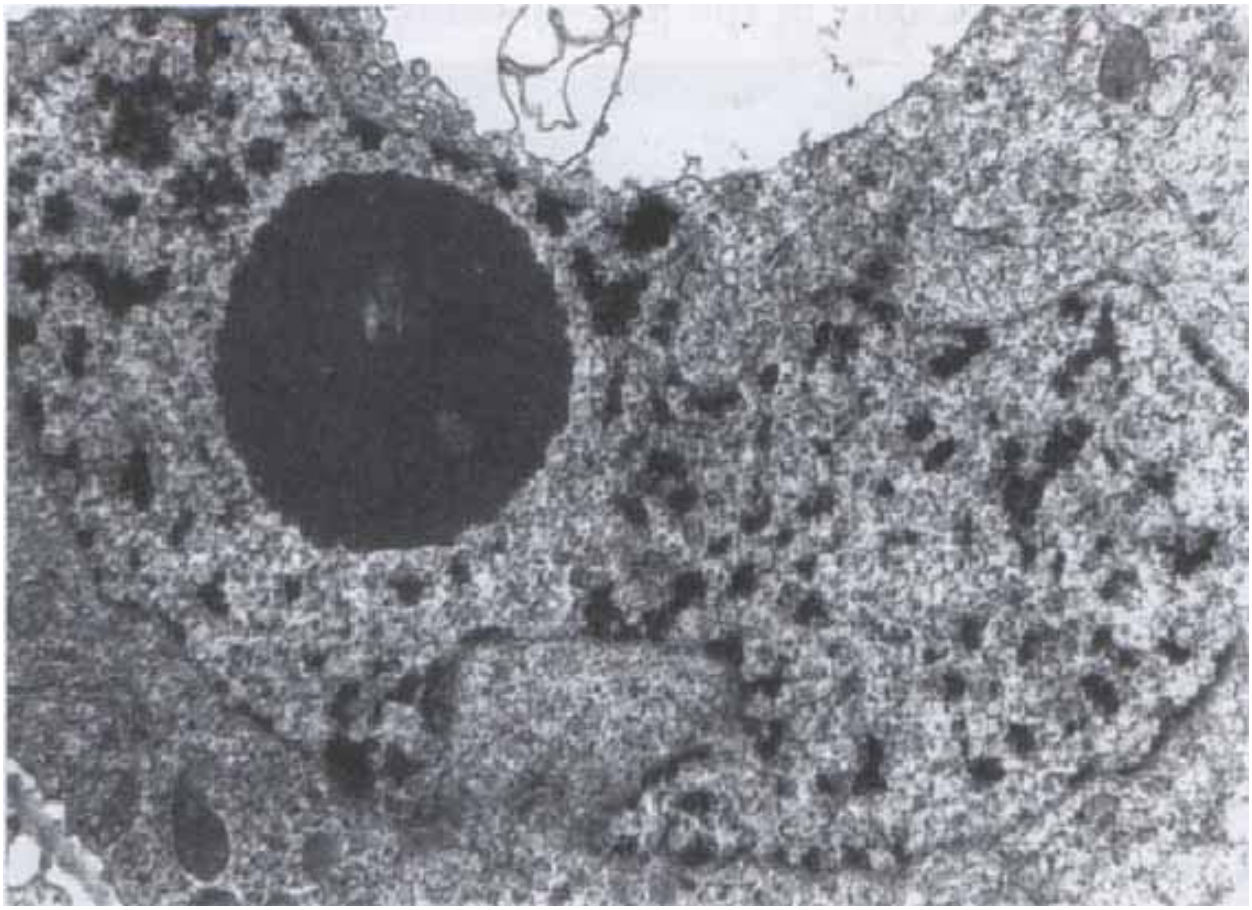
x 50, 000

Figure 52. Fragment of a root cell of sunflower after 10 min incubation in 10^{-3}M solution of [1^{14}C] phenoxyacetic acid. Penetration of the label into different cell organelles is visible.



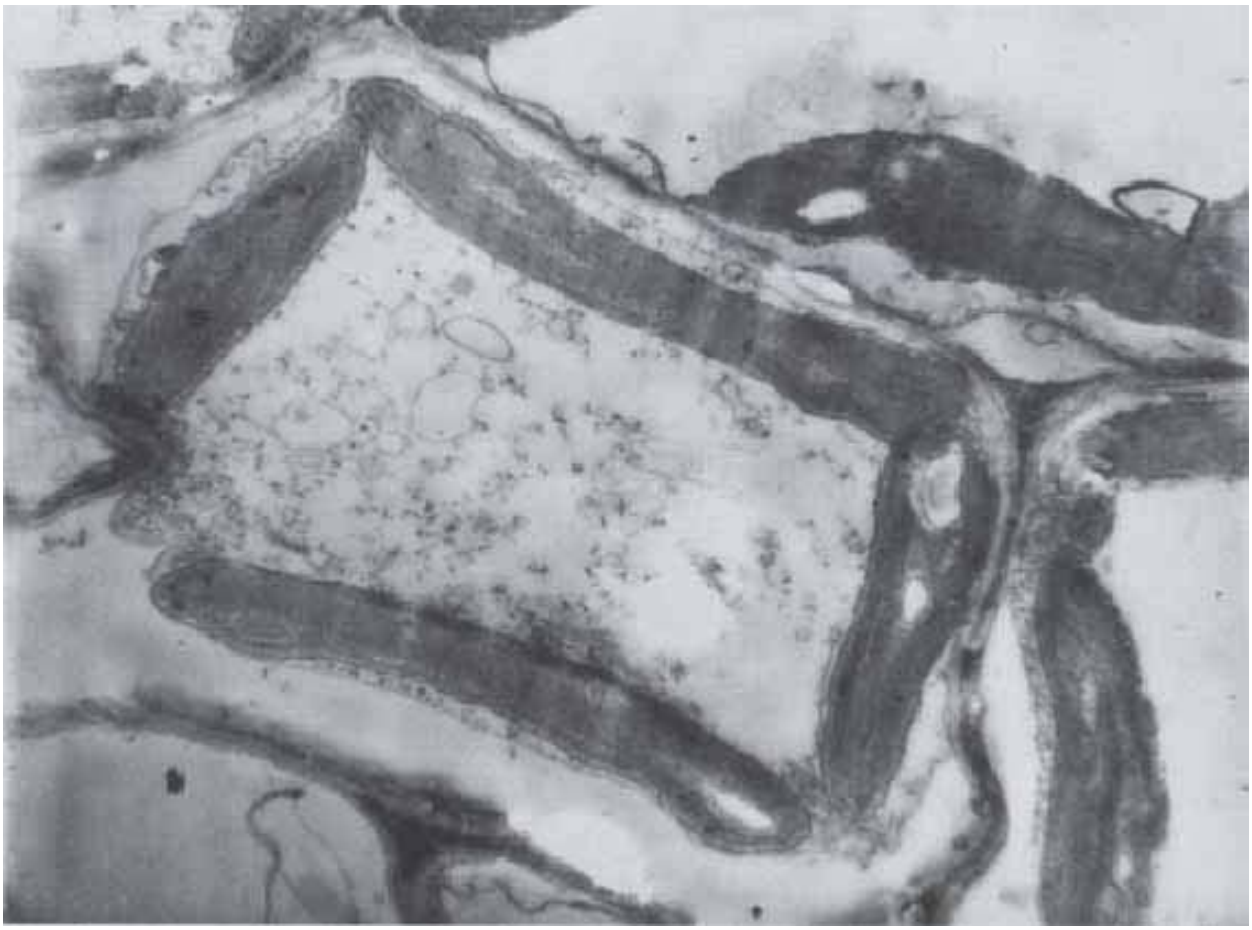
x 25, 000

Figure 53. Fragment of soybean root cell after 10 min incubation in 1.5 $x10^{-3}$M solution of nitrobenzene. Mitochondria with swollen crista are visible..



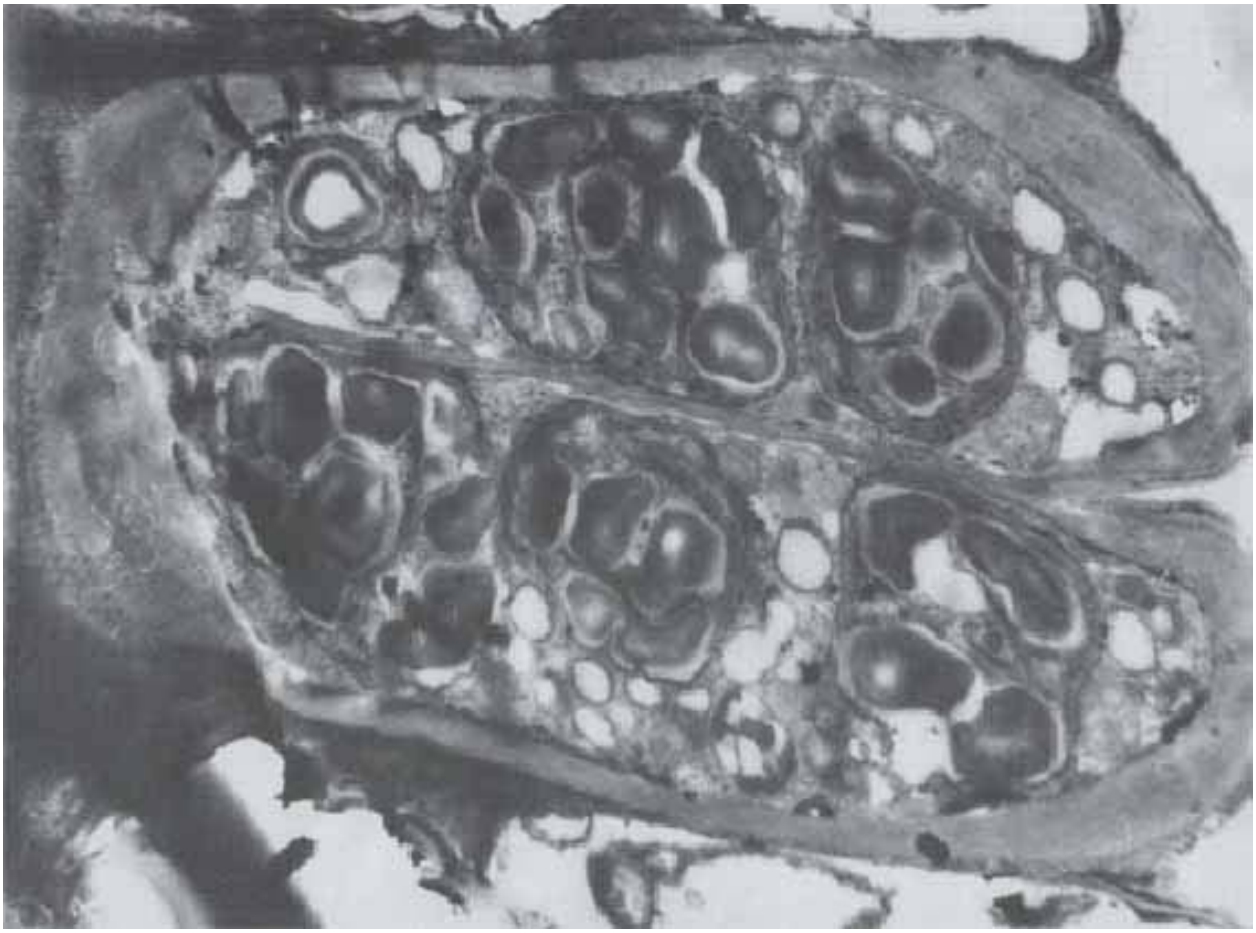
x 60, 000

Figure 54 . Fragment of maize root cell incubated in benzidine solution (2.25 $x10^{-3}$M). Note the intensive invagination of the nuclear membrane.



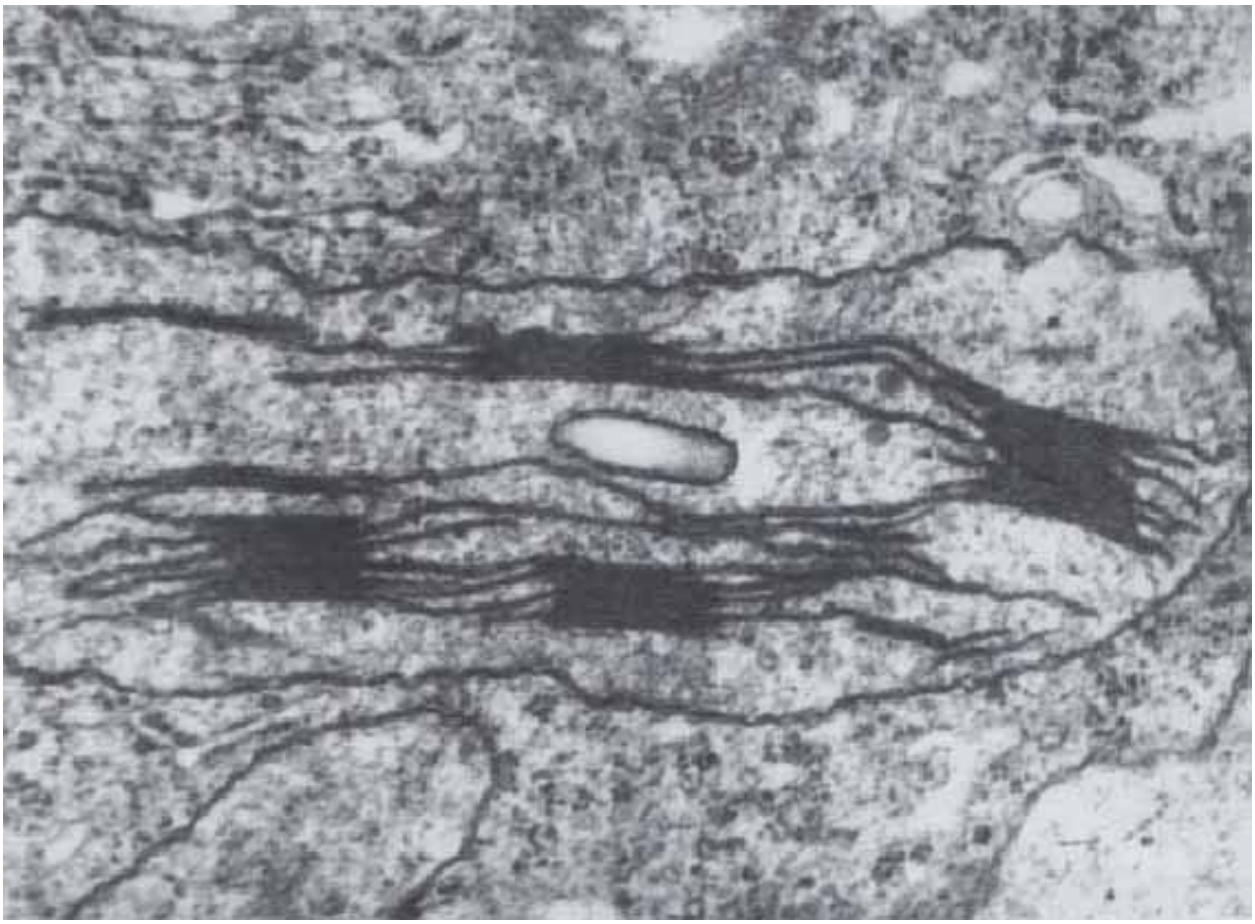
x 30, 000

Figure 55. The chloroplasts in epidermal cells of maize leaves exposed to methane. The changes in shape of the chloroplasts are visible.



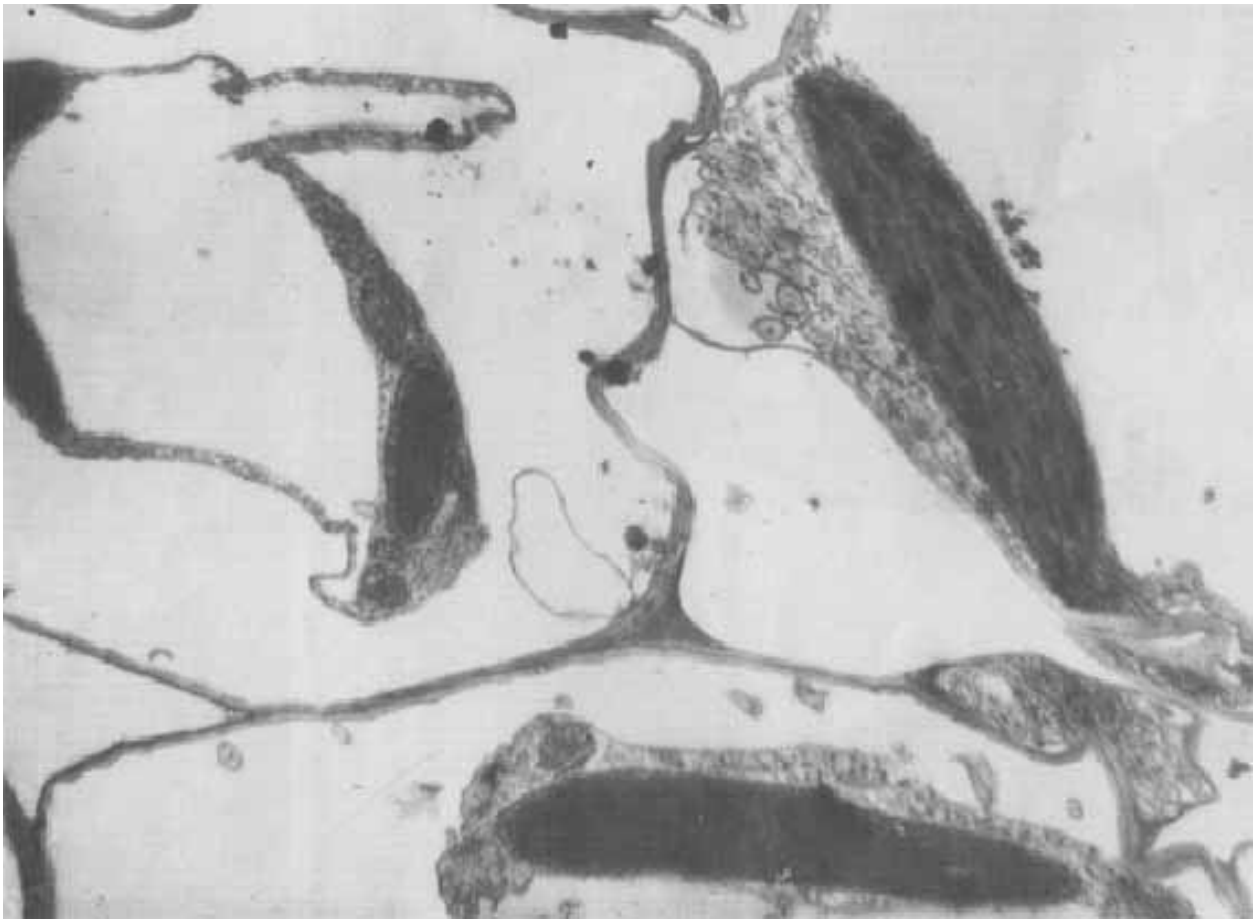
x 30, 000

Figure 56. The chloroplasts in epidermal cells of maize leaves exposed to ethane. Large quantities of starch granules are visible.



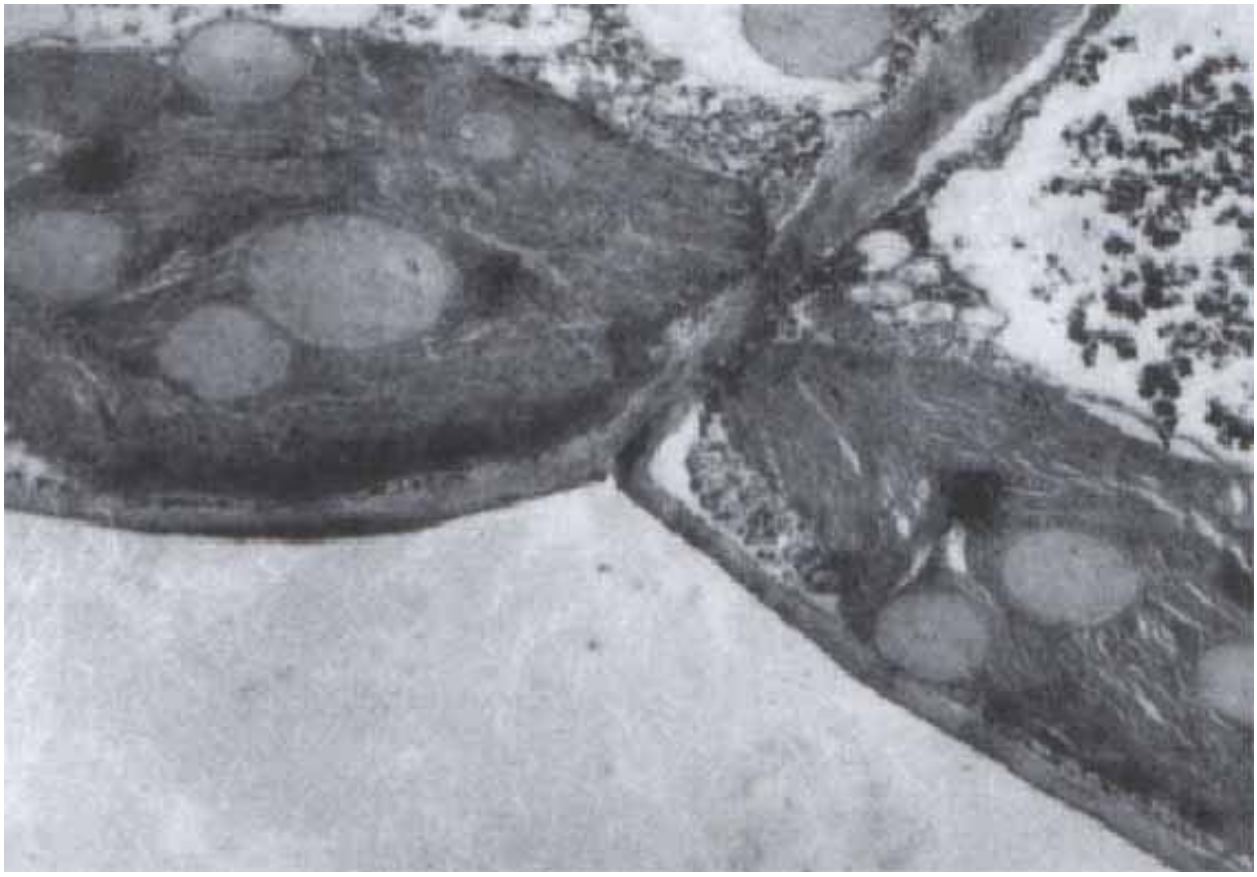
x ,30, 000

Figure 57. The chloroplasts in cells at the lower side of maize leaves exposed to ethane. Large quantities of starch granules are visible.



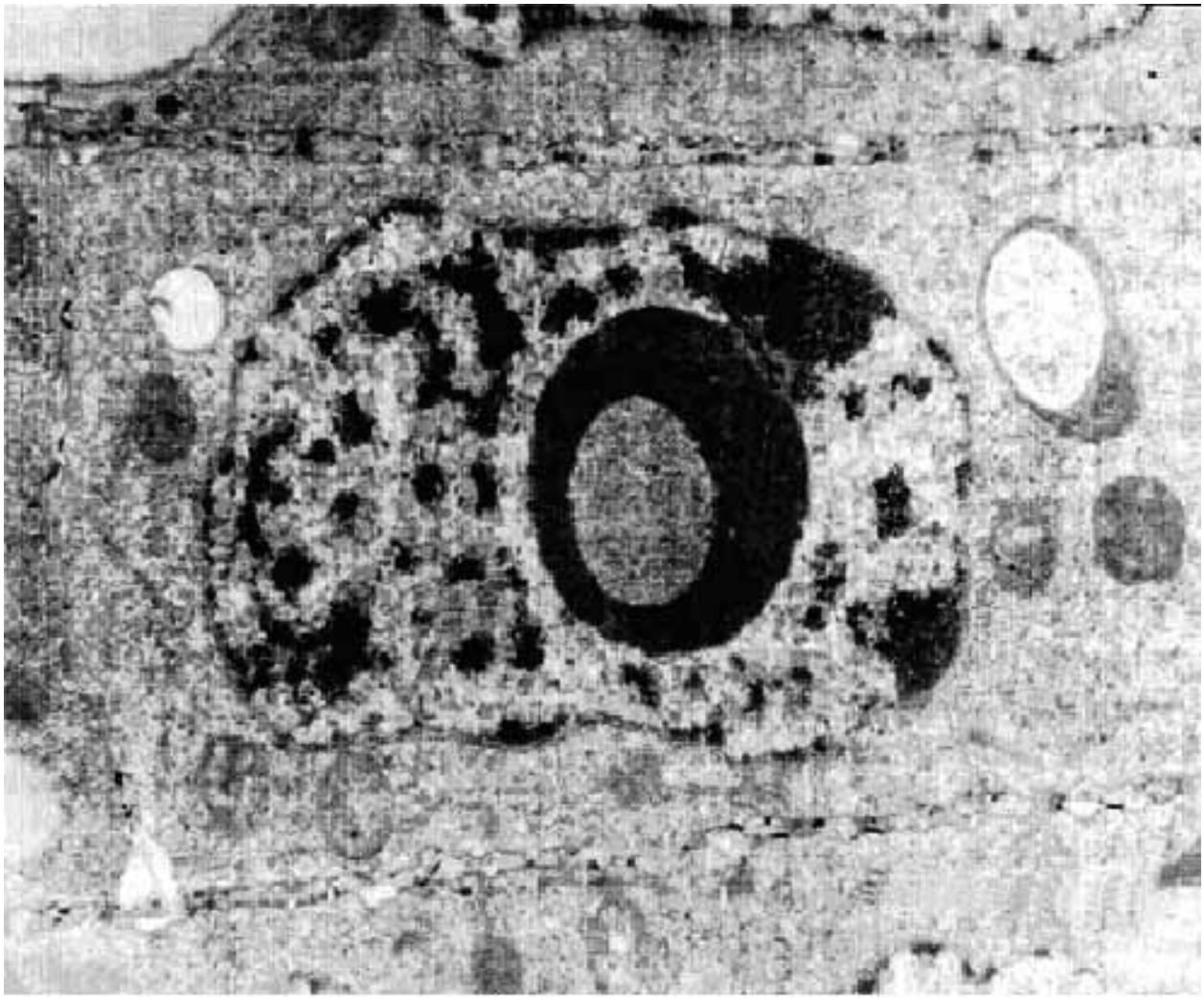
x 30, 000

Figure 58. The chloroplasts in cells of maize leaves exposed to a mixture composed by methane 88.7%, ethane 6.8%, propane 2.8% and butane 1.7%. Elongation of the chloroplasts, chaoticdislocation of grains and cell destruction are visible.



x 60, 000

Figure 59. A fragment of a maple leaf incubated in [l,6^{14}C] benzene (10^{-4}M). Note the change in structure of the photosynthetic apparatus and accumulation of inclusions insertions.



x 60, 000

Figure 60. A fragment of a maize root cell after incubation in a solution containing 10^{-3}M solution of 3,4-benz[a]pyrene.

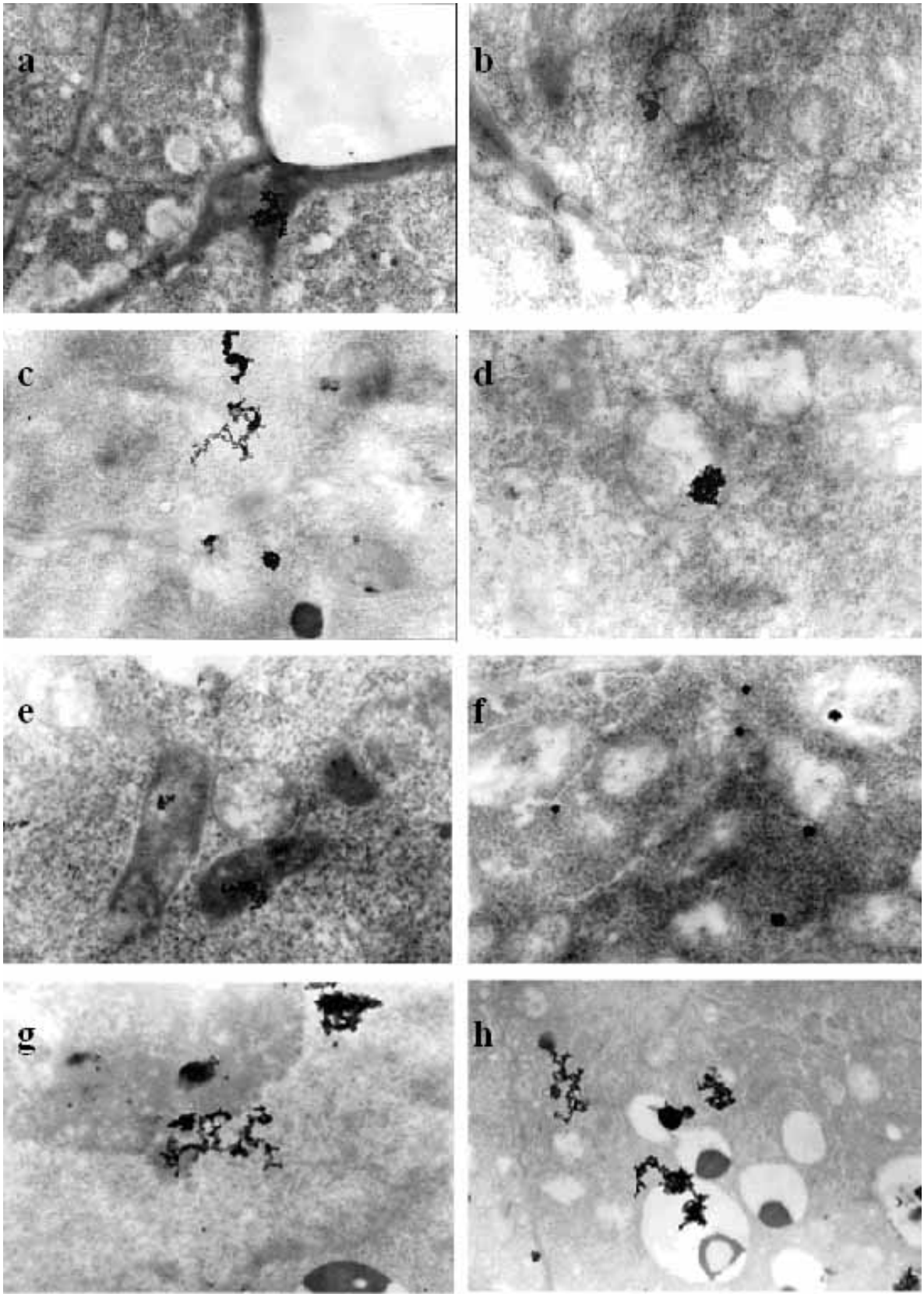

a
b
c
d
e
f
g
h

Figure 61. Cortical root cells of soybean seedlings, grown on 0.5 mM (1-^{14}C) TNT.
a) Label in cell wall.
b) Label in contact with the outer membranes of the mitochondria.
c) Label in the mitochondria, on the plasmalemma, in the endoplasmic reticulum.
d) Label in the mitochondria.
e) Label in the plastids.
f) Label in the mitochondria and endoplasmic reticulum.
g) Label in nucleus and nucleoli.
h) Label in vacuoles, plastids and mitochondria.

a **b**

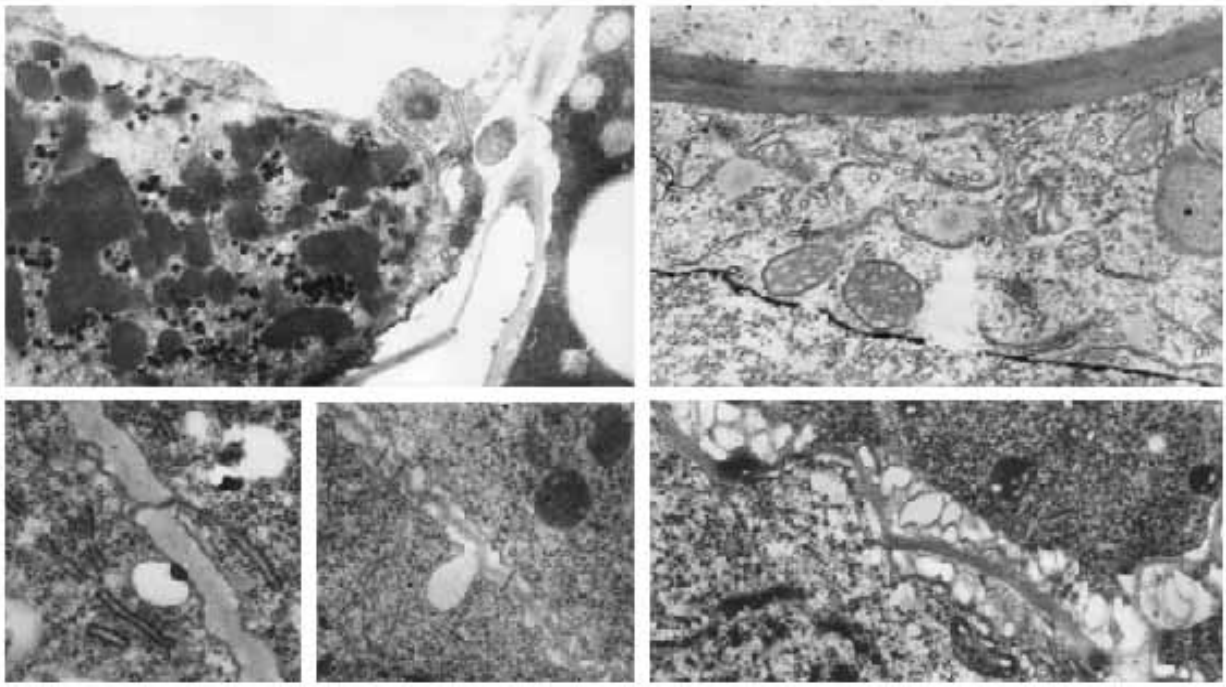


Figure 62. Fragments of root apex cells of maize seedlings after incubation with toxicants.

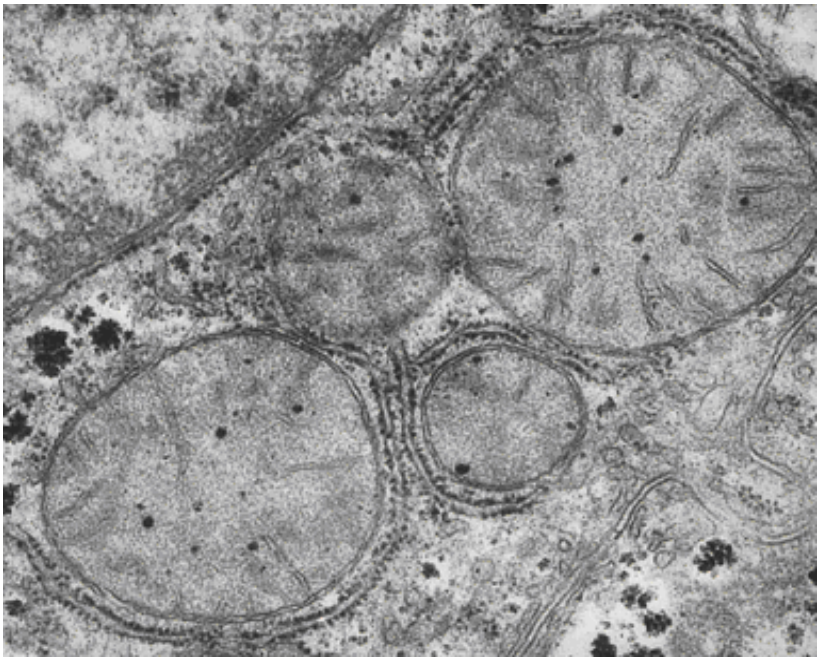
a) Contact between the endoplasmic reticulum and the vacoules.

b) Fragmentation of a granulated endoplasmic reticulum.

c) Contacts between vesicles and the plasmalemma.

d) Junction of vesicule mebranes with plasmalemme.

e) Membrane fragments accumulated in the periplasma.



x 60 000

Fig.63. Fragment of cell of maize root apex after incubation with $1.5 \cdot 10^{-4}$M solution of nitrobenzene. Large amount of mitochondria surrounded by membranes of endoplasmic reticulum is observed.